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From Insight to Outcome

High Throughput Quantitative Analysis

MassHunter Quantitative Analysis Webinar Series

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Agilent

MassHunter Quantitative Analysis Software

Review and Quant Method Optimization

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What is quantitative analysis?

In analytical chemistry, quantitative analysis is the determination of the absolute or relative abundance (often expressed as a concentration) of one, several or all particular substance(s) present in a sample.

From https://en.wikipedia.org/wiki/Quantitative_analysis_(chemistry)

Quantitative chemical analysis, branch of chemistry that deals with the determination of the amount or percentage of one or more constituents of a sample.

From https://www.britannica.com/science/quantitative-chemical-analysis

Quantitative analysis refers to the determination of how much of a given component is present in a sample.

From https://www.thoughtco.com/definition-of-quantitative-analysis-604627

How do I know that the compound that is identified is indeed the correct compound?'

Target Compound Analysis

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Retention Time	• A given compound will come out at a specified time under a given set of chromatographic conditions. • Dual column analysis with columns of different polarities.
Target Ion	• Must be unique to the compound of interest in the time range of interest.
Qualifier Ions	• Must be present in the same specified time range. • Must be present in specific ratios relative to the Target Ion.
Target and Qualifiers	• Must be within the given correlation window. • Should maintain similar peak shapes.

Increasing Confidence

Analysts are buried in data!!!

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Analysts are being overwhelmed with data … hundreds of compounds… multiple signals per compound…10's or even hundreds of samples in a batch.

Add an initial calibration, a continuing calibration or a QC and other data review functions and the analyst is overwhelmed with data.

Chromatographic runs are shorter; thus more data in a given period of time.

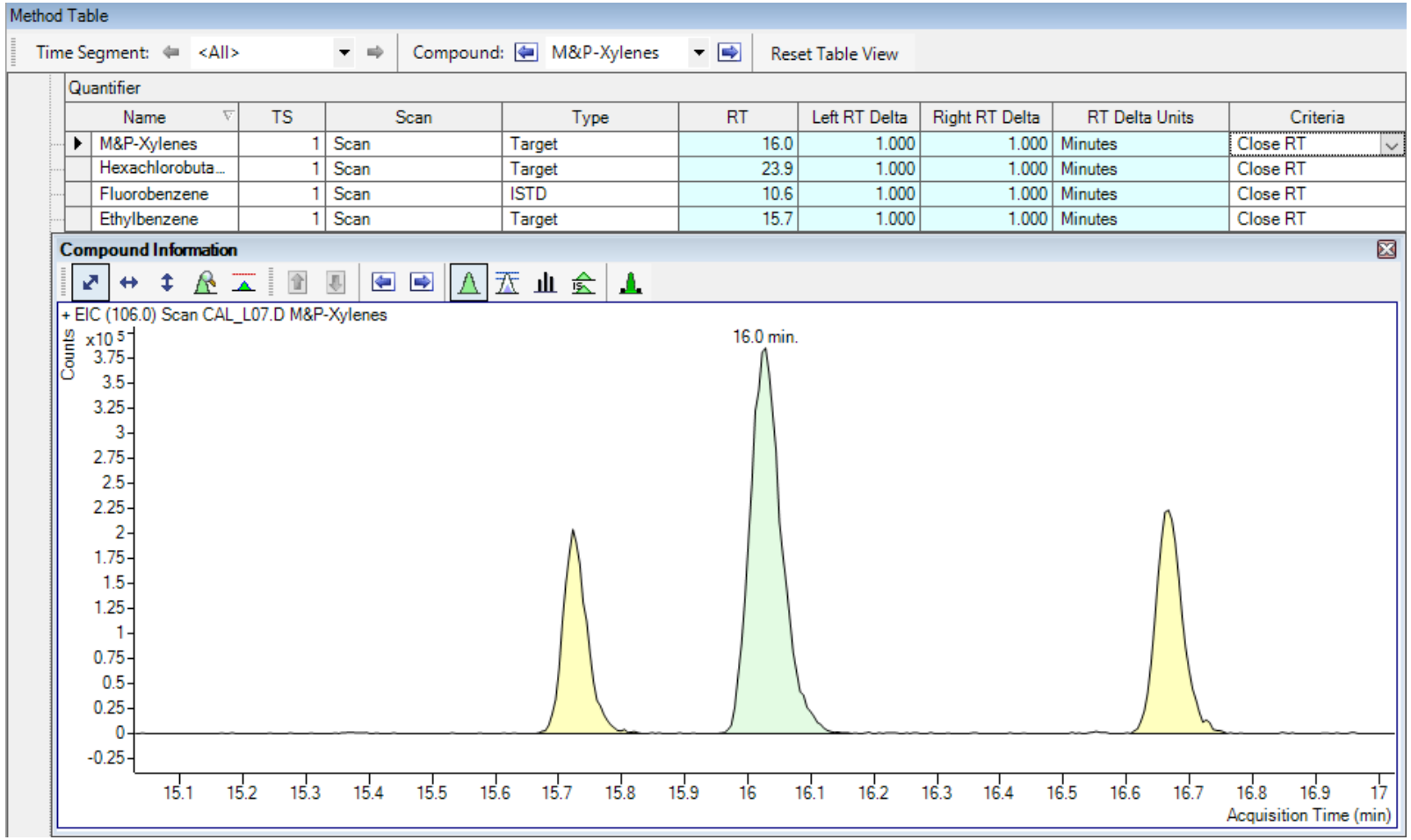
What can what can MassHunter Quantitative Analysis software do to help?

Quant Method Check List

- Extract the compounds signals in the correct RT Window.
- Utilize the correct integrator and settings for each compound.
- Reduce unwanted peaks with
  - Reference and Non Reference Windows
  - Integration parameters
    - Peak Filters
    - Peak Filter Area Threshold
    - Zero Peaks Below LOD
    - Correlation Window
- Updating Retention Time Drift
- Retention Times
- Qualifier Ion Ratios
- Update Mass Assignments

Objective is to intelligently minimize the number of compounds that require review.

Retention Time Setup
RT Delta



Left and Right RT Delta determines the time range over which the specified signal is extracted.

RT Delta Units

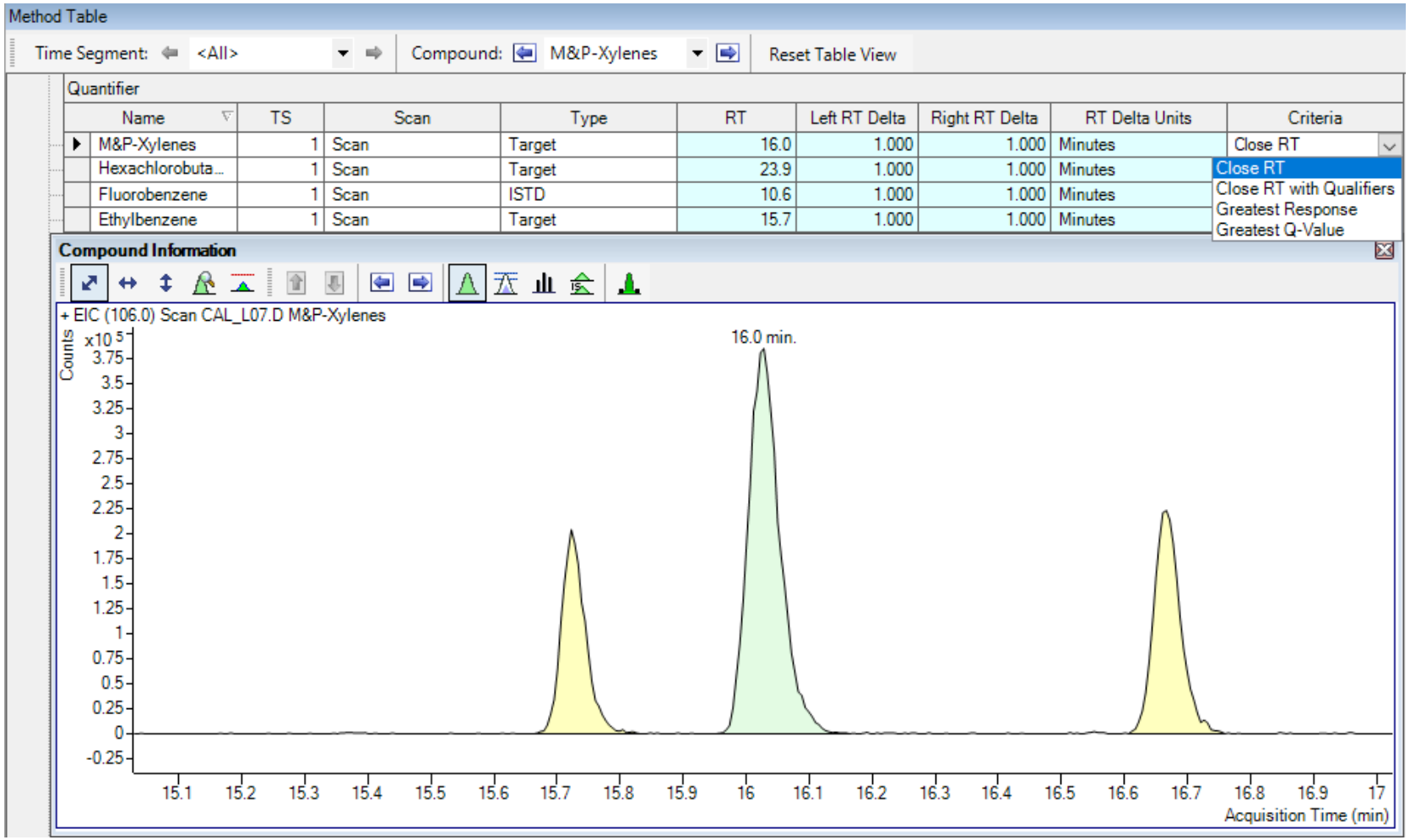
- **Percent** – Uses a percentage of RT, good for long runs where retention time shifts can be larger late in the run.
- **Minutes** - Absolute minutes.

Default is 1 minute. A narrower window can be setup if needed.

Retention Time Setup
Criteria

When multiple peaks are found in RT window, Criteria decides which peak to use.

- Close RT
- Close RT with Qualifiers
- Greatest Response
- Greatest Q-Value



Reference & Non Reference Window

Definitions

Defined in Globals Setup in the Method Editor

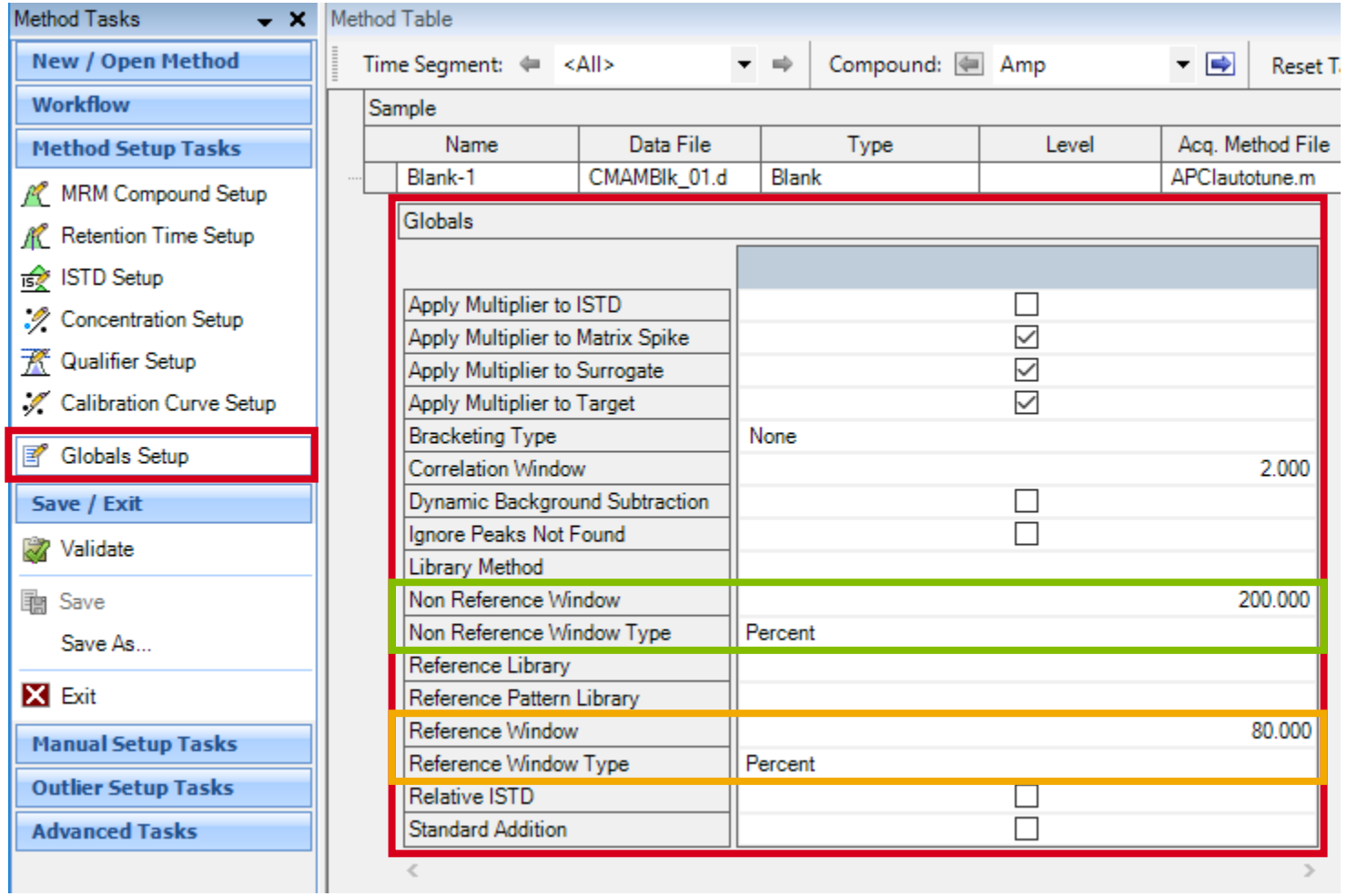
Reference Window

- Applies only to compounds labeled as Time Reference.
- Only ISTDs can be labeled as Time Reference.
- Algorithm looks for ISTDs first, then target compounds related to that ISTD.

Non Reference Window

- Applies to all other compounds.

Recognition and Reference Windows are synonymous terms.



Reference & Non Reference Window

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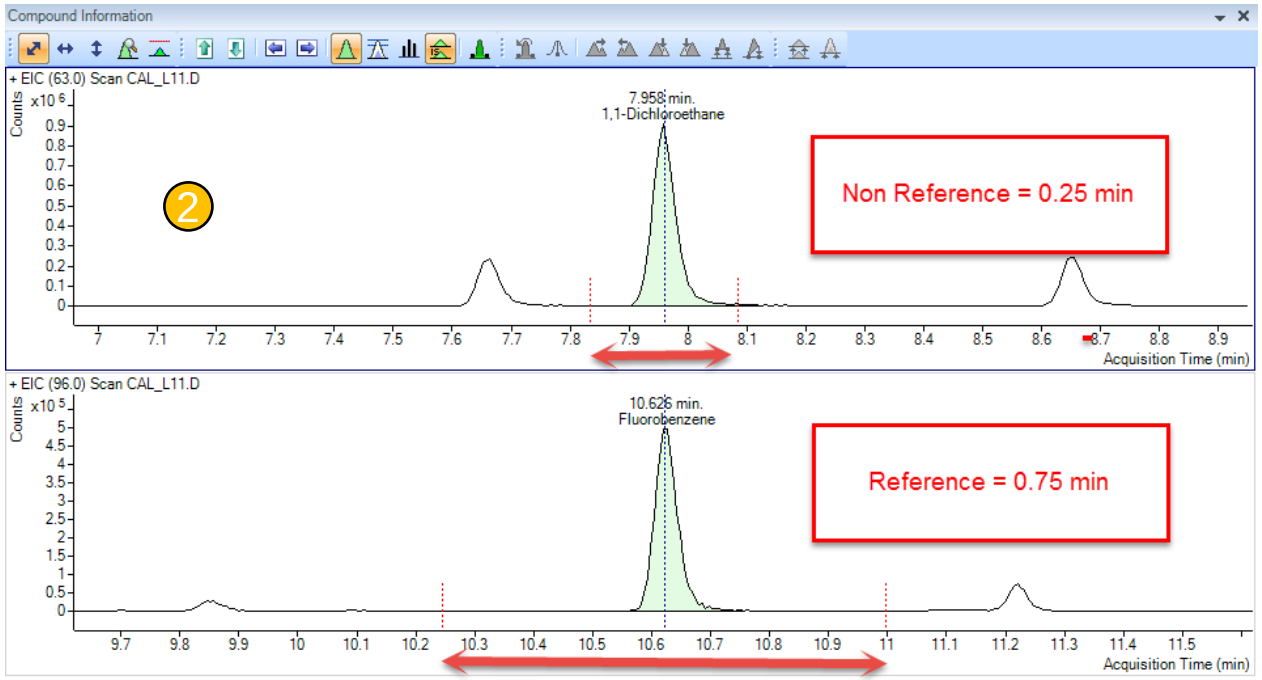
1

Globals	
Apply Multiplier to ISTD	☐
Apply Multiplier to Matrix Spike	☑
Apply Multiplier to Surrogate	☑
Apply Multiplier to Target	☑
Bracketing Type	None
CC Maximum Elapsed Time In Hours	0.000
Correlation Window	2.000
Dynamic Background Subtraction	☐
Ignore Peaks Not Found	☐
Library Method	
Non Reference Window	0.250
Non Reference Window Type	Minutes
Reference Library	
Reference Pattern Library	
Reference Window	0.750
Reference Window Type	Minutes
Relative ISTD	☐
Standard Addition	☐

Restricts peak selection to a smaller RT window.



3

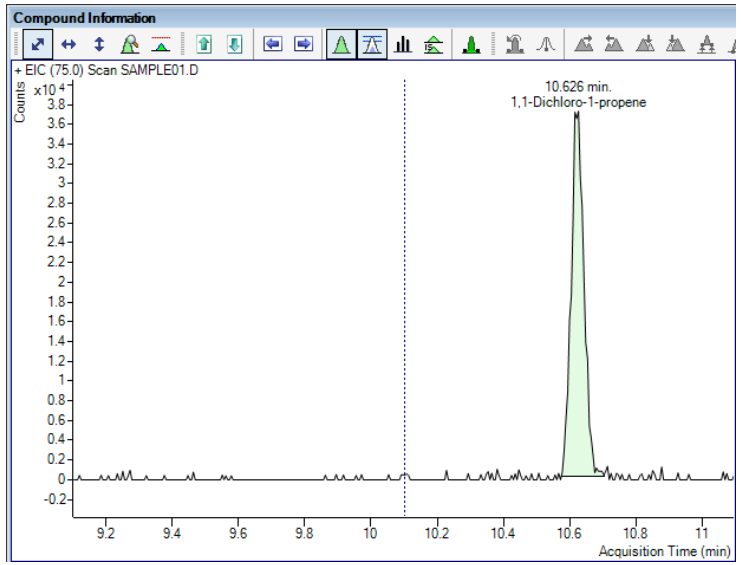
Quantifier

Name	TS	Scan	Type	ISTD Compound Name	ISTD Flag	ISTD Conc.	Time Reference Flag
Fluorobenzene	1	Scan	ISTD	<None>	☑	10.0000	☑
Chlorobenzene-d5	1	Scan	ISTD	<None>	☑	10.0000	☐
1,4-Dichlorobenzene-d4	1	Scan	ISTD	<None>	☑	10.0000	☐
1,2-Dichloroethane-d4	1	Scan	Surrogate	Fluorobenzene	☐	10.0000	☐
Toluene-D8	1	Scan	Surrogate	Chlorobenzene-d5	☐	10.0000	☐
1,1-Dichloro-1-propene	1	Scan	Target	Fluorobenzene	☐	10.0000	☐
Dichlorodifluoromethane	1	Scan	Target	Fluorobenzene	☐	10.0000	☐
Chloromethane	1	Scan	Target	Fluorobenzene	☐	10.0000	☐

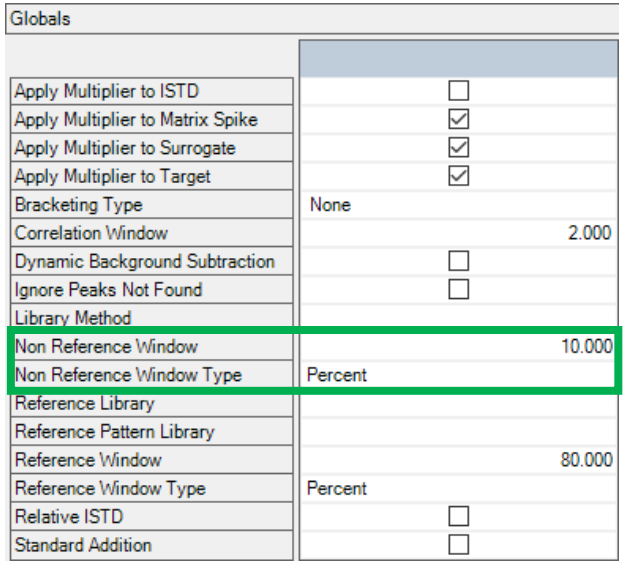
Reference = ISTD + Time Reference

Non Reference = Everything else

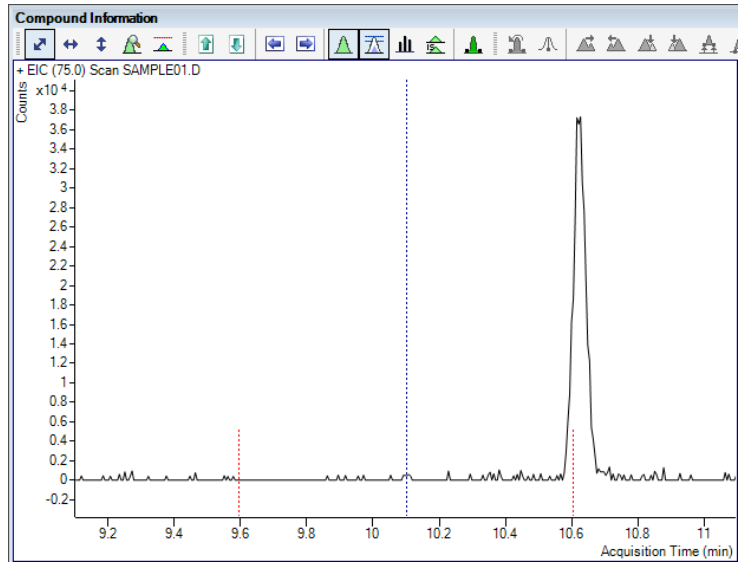
Non Reference Window



Initial Values



Adjusted Values

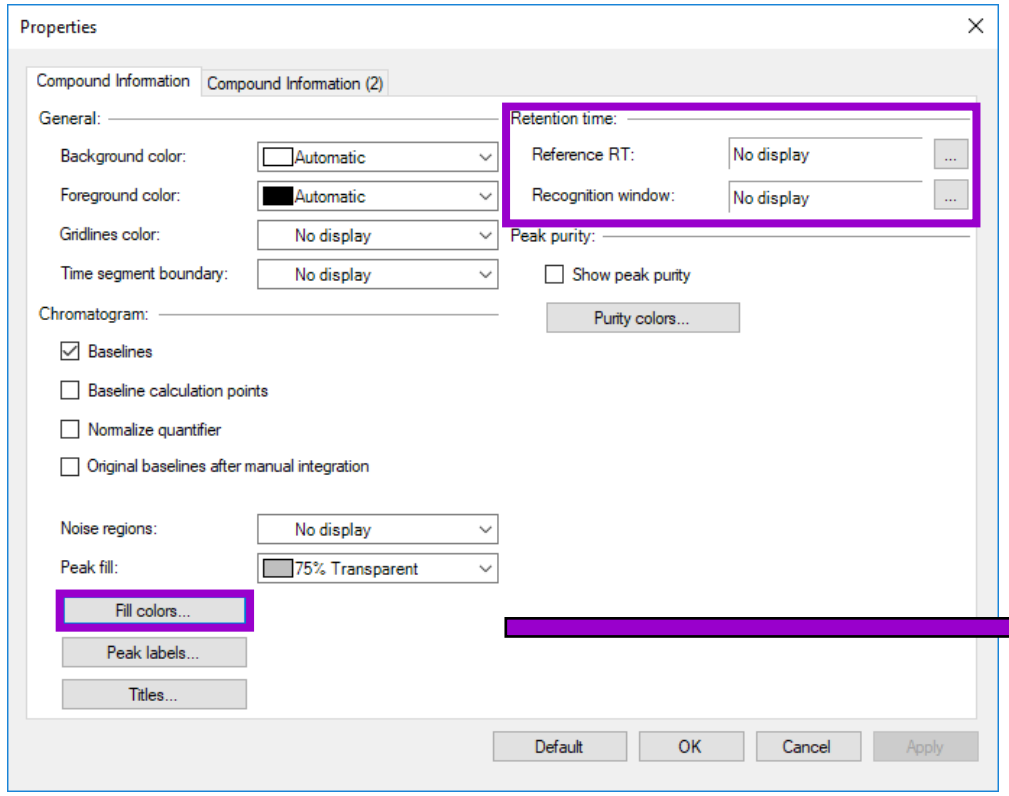


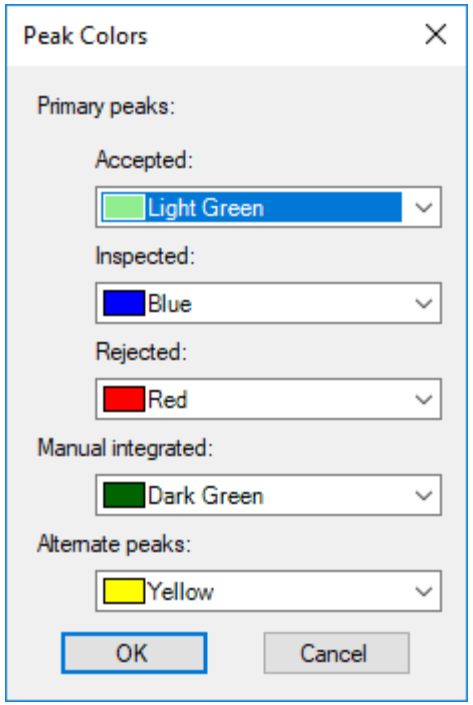
Final Values

Reduce the Non Reference Window from the default 200% to 10%. This helps eliminate false positives and reduced data review and “zero peak” work.

Or, if you prefer, switch to absolute minutes. Though keep in mind this setting is for **all** peaks in the quantitation method and cannot be changed on a compound by compound basis.

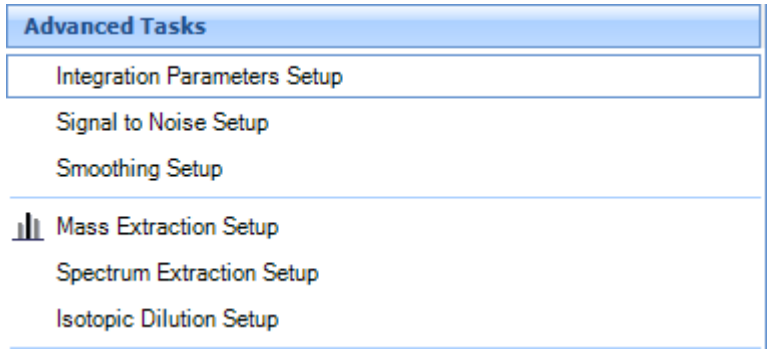
Properties Window

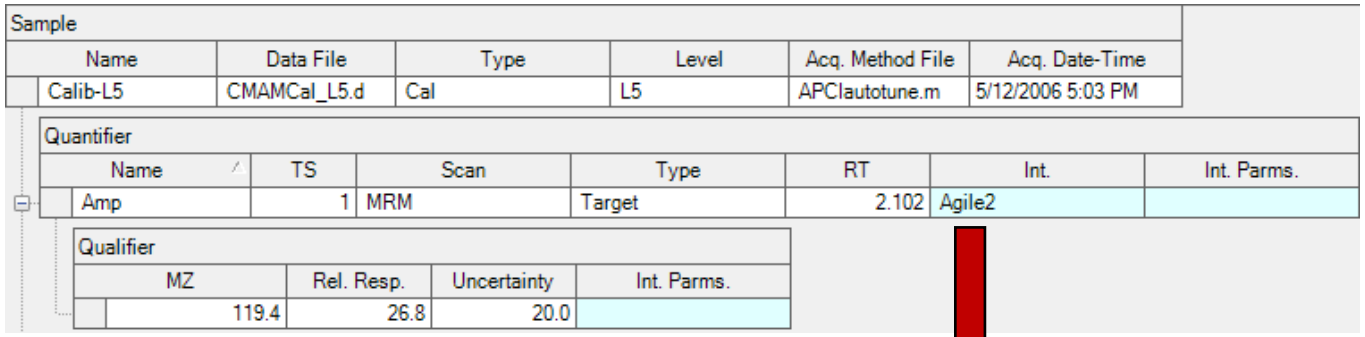


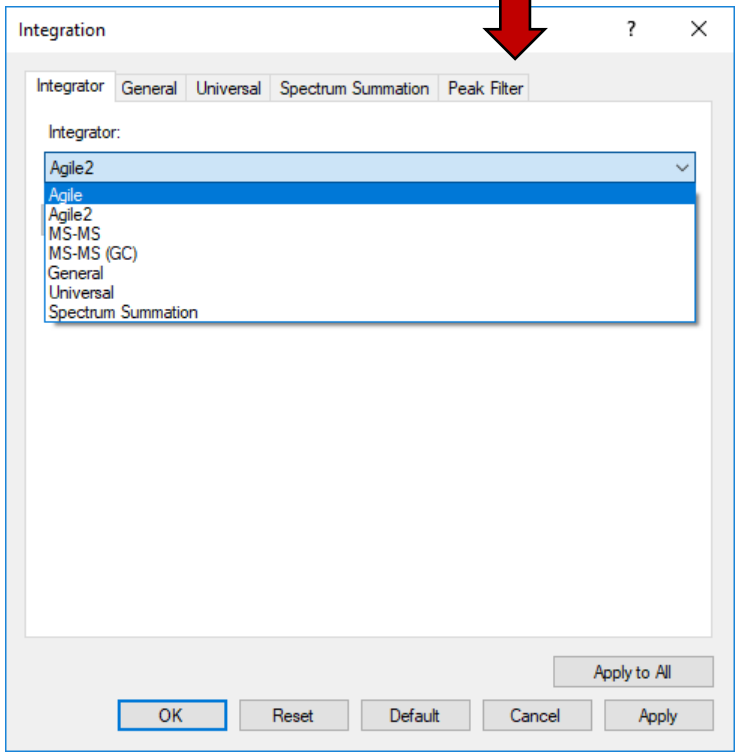


Right click **Properties > Fill Colors.**

Integrators
Choose the Right Integrator







- **Method >Edit >Advanced Tasks > Integration Parameters Setup**
- Each compound can have its own integrator.
- Choose the one best suited for the compound's chromatography.
- Start with Agile2 (parameter-less) and move to other integrators if they work better.
- **All** integrators use Peak Filters.

Integrators

Agile2

- 3rd generation parameter-less integrator
- Default Integrator
- Better baselines, higher sensitivity to smaller peaks

Agile

- 2nd generation parameter-less integrator

Universal

- 1st generation ChemStation integrator
- Familiar to GC LC ChemStation users

General (RTE)

- Familiar to MSD ChemStation users
- Areas in Universal are 10 time smaller than seen in ChemStation.

MS/MS and MS/MS (GC)

- 1st generation parameter-less integrator intended for MS/MS systems, not recommended for SQ. Originally required 64 data points.

ChemStation

- 2nd generation ChemStation
- Intended for UV

Integrators
General, Universal, Spectrum Summation

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Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
Calib-L5	CMAMCal_L5.d	Cal	L5	APClautotune.m	5/12/2006 5:03 PM

Quantifier

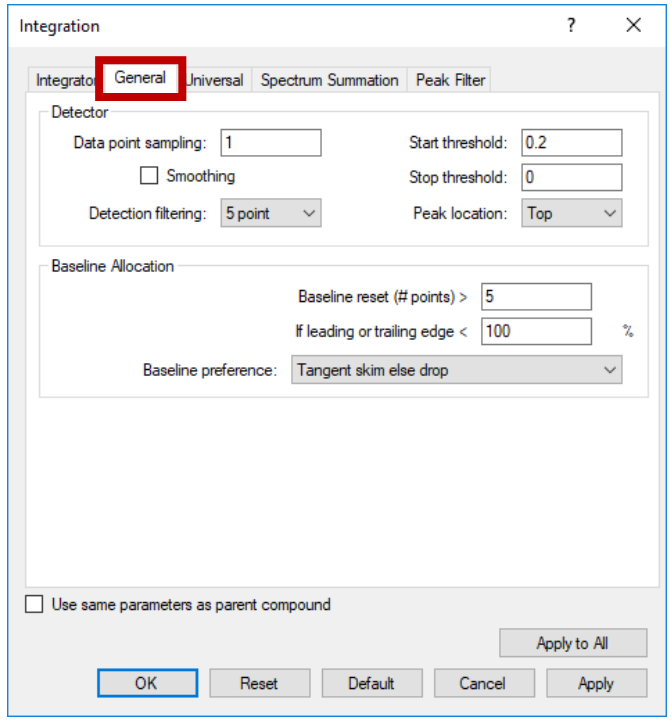
Name	TS	Scan	Type	RT	Int.	Int. Parms.
Amp	1	MRM	Target	2.102	Agile2	

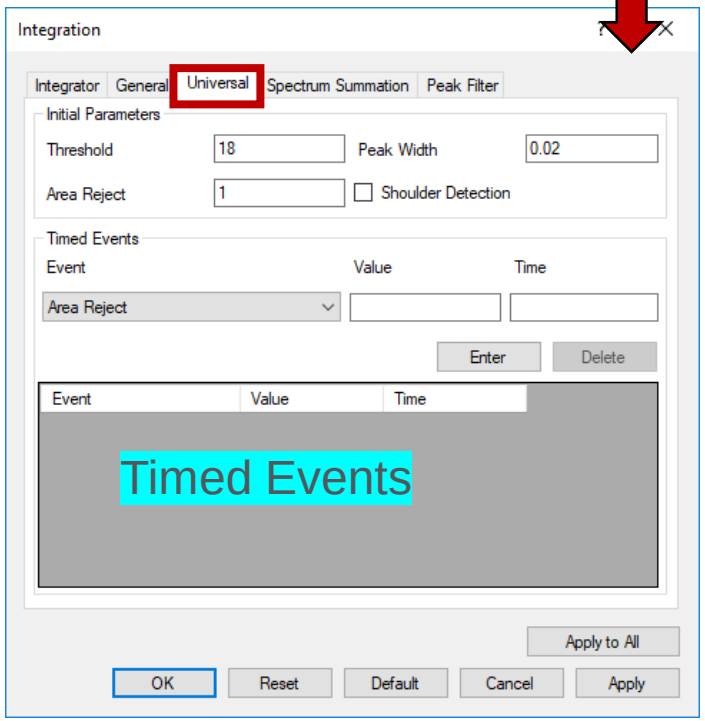
Qualifier

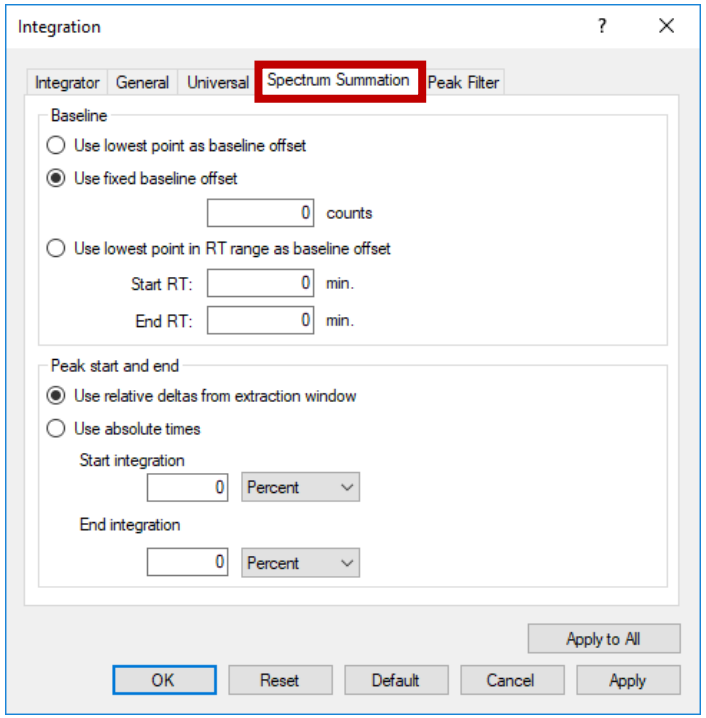
MZ	Rel. Resp.	Uncertainty	Int. Parms.
119.4	26.8	20.0	

Adjust parameters as needed for each compound to get optimal integrations.

Tip: Evaluate low and high level calibrators.







Integrators
Qualifier Integration Control

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Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
Calib-L5	CMAMCal_L5.d	Cal	L5	APClautotune.m	5/12/2006 2:03...

Quantifier

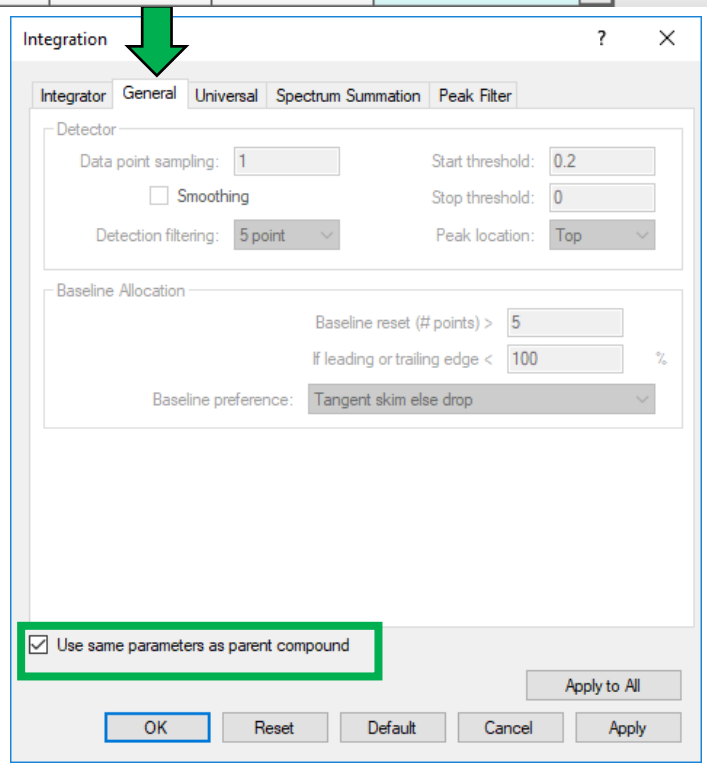
Name	TS	Transition	Scan	Type	RT	Int.	Int. Parms.
Amp	1	136.2 -> 91.4	MRM	Target	2.102	General	...

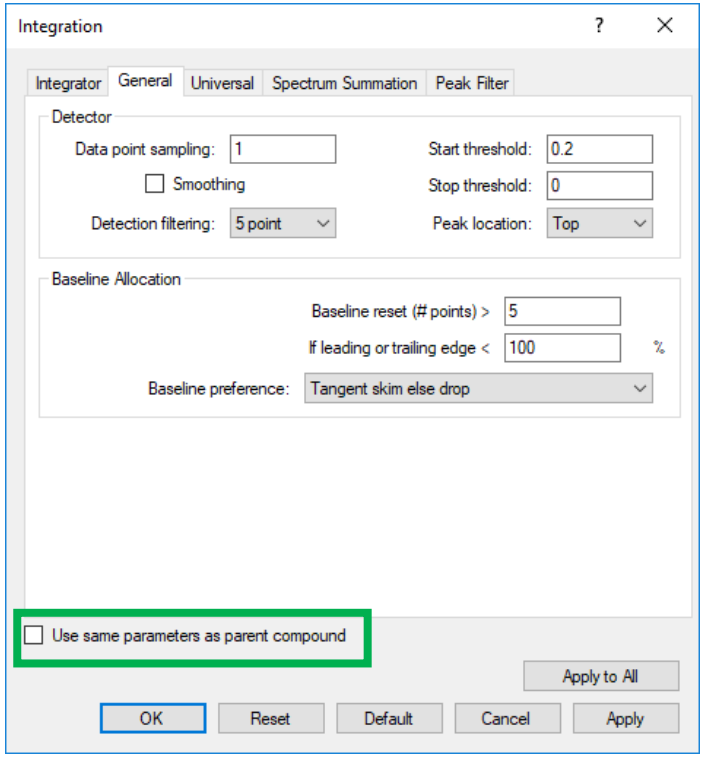
Qualifier

Precursor Ion	Product Ion	Transition	Rel. Resp.	Uncertainty	Int. Parms.
136.2	119.4	136.2 -> 119.4	26.8	20.0	

Qualifiers use the same integrator as quantifier.

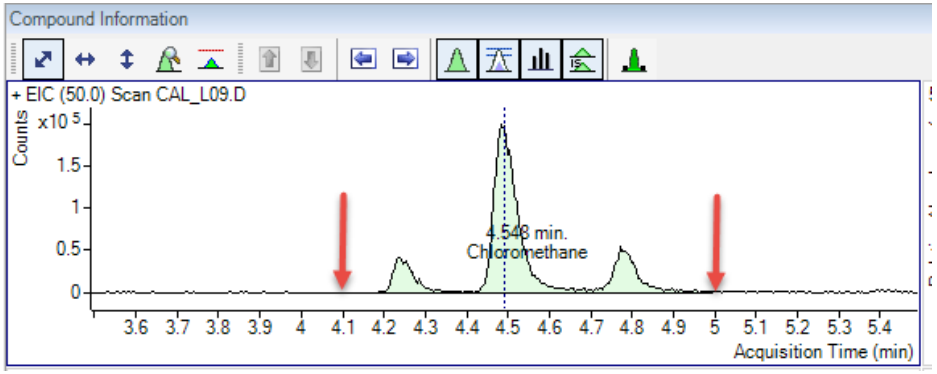
If needed each qualifier may have separate parameters to optimize integration but the default is to use quantifier parameters.

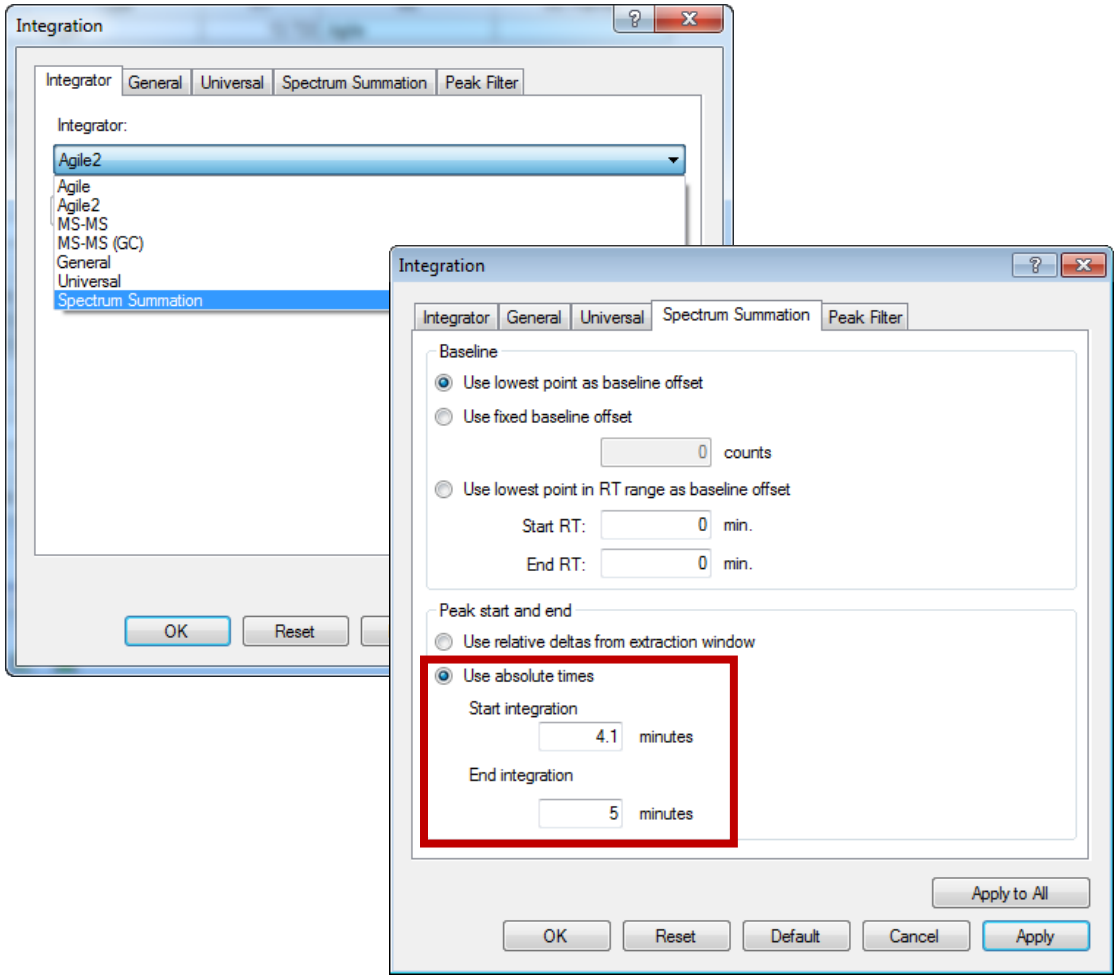




Spectrum Summation Integrator

- Integrator designed for situations where compounds are poorly separated or peak shape is highly irregular such as ….
  - PCB mixtures
  - Fraction cut in hydrocarbons
  - Flow injection analysis (FIA)
- Sums signal over a time range.
- Exclude signal below threshold.
- Always gives a horizontal baseline.
- RT reported as the center of the time range.





Integrators
Peak Filters

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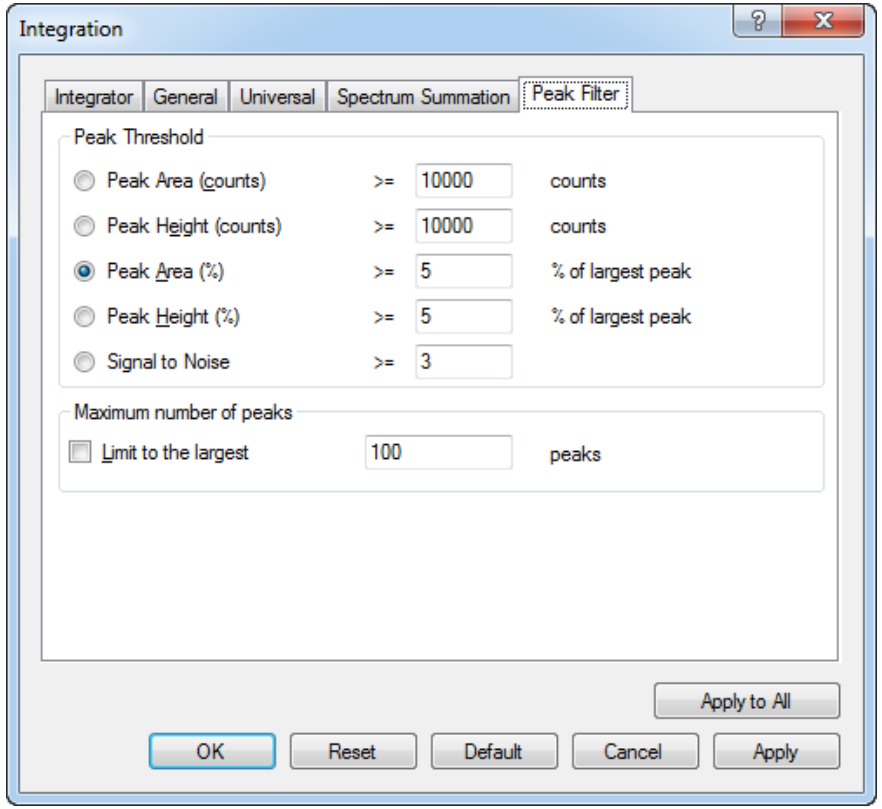
- Available on all integrators including parameter-less ones.
- Separate Peak Filters for quantifier and qualifiers.
- Peak Filter is run after integrators create a peak list and then removes peak based on settings.
- Ideal to automatically remove small peaks that would otherwise require manual review and correction.

Default Setting for both Target and Qualifiers is Peak Area >= 5 % of largest peak.

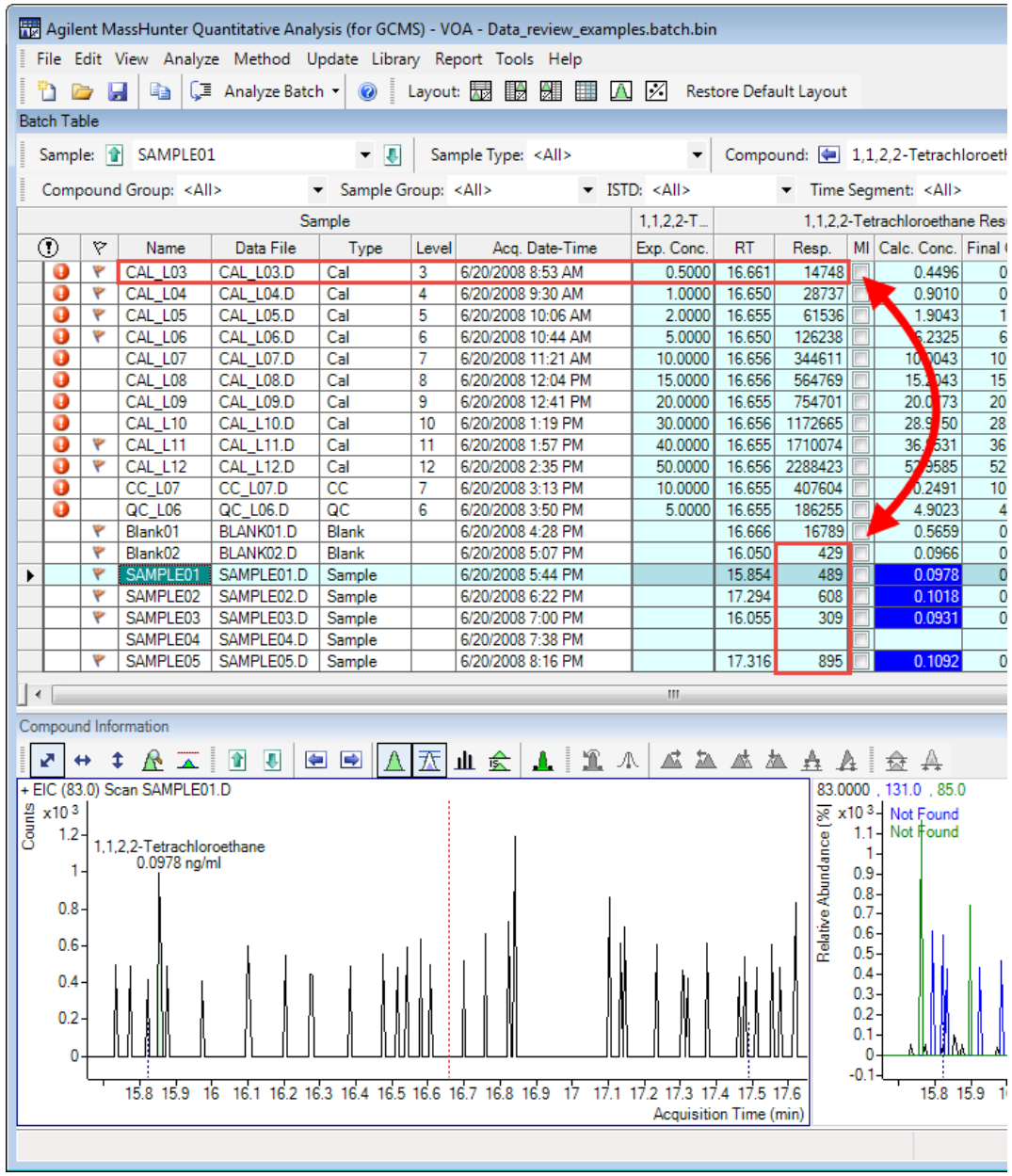


Peak Filter
Area

Typical noise or matrix peaks may be picked up by the integrator.

They are far too small relative to the response of the lowest level Calibrator to be reported. Normally we would need to "zero peak" each one.

Instead we can use peak filters in the method to remove the unwanted peaks.



Adjusting Peak Filter Area Thresholds
Manual

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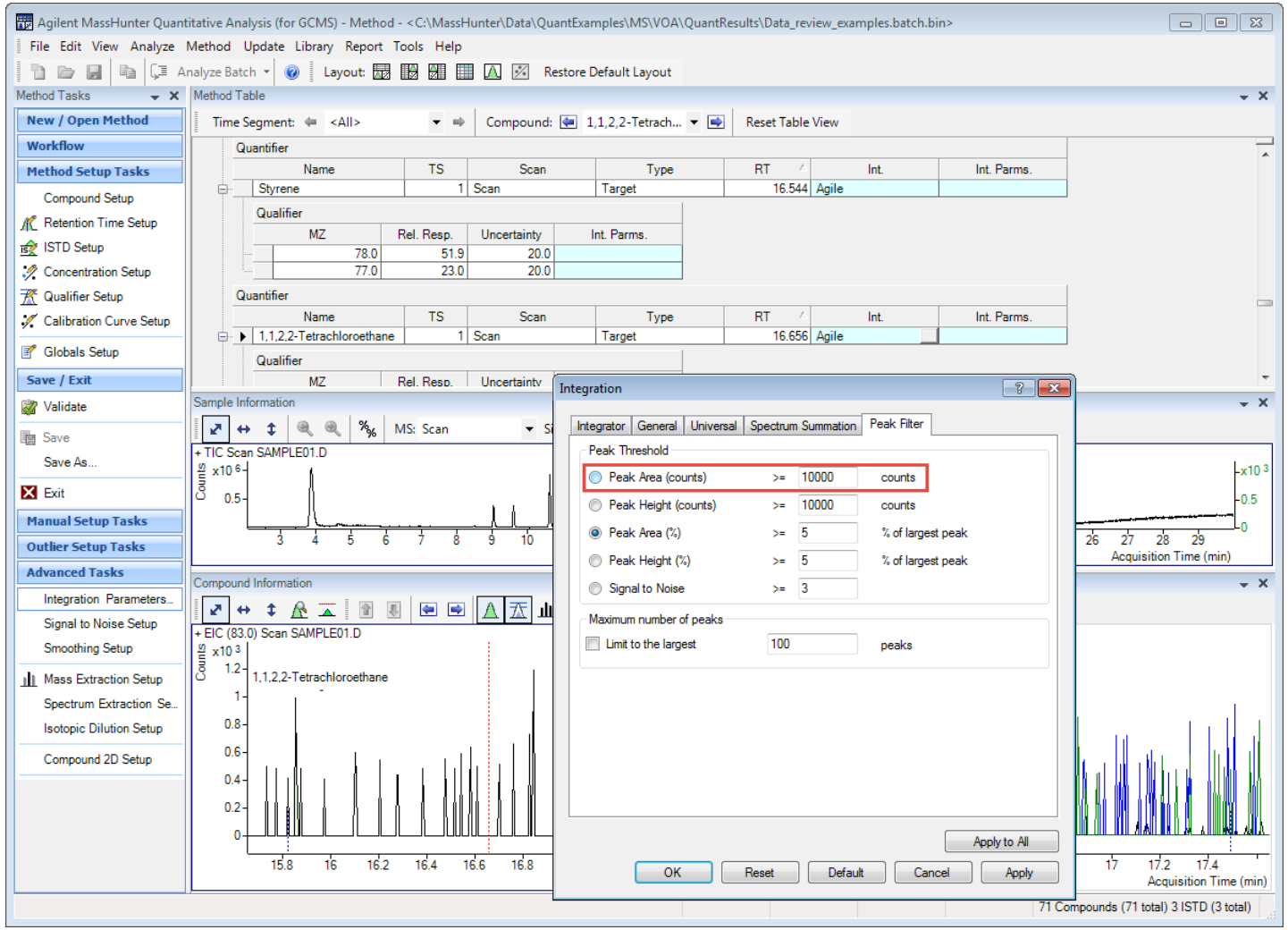
Setting the peak area threshold for each compound would be a slow and tedious process…



Adjusting Peak Filter Area Thresholds
Automated

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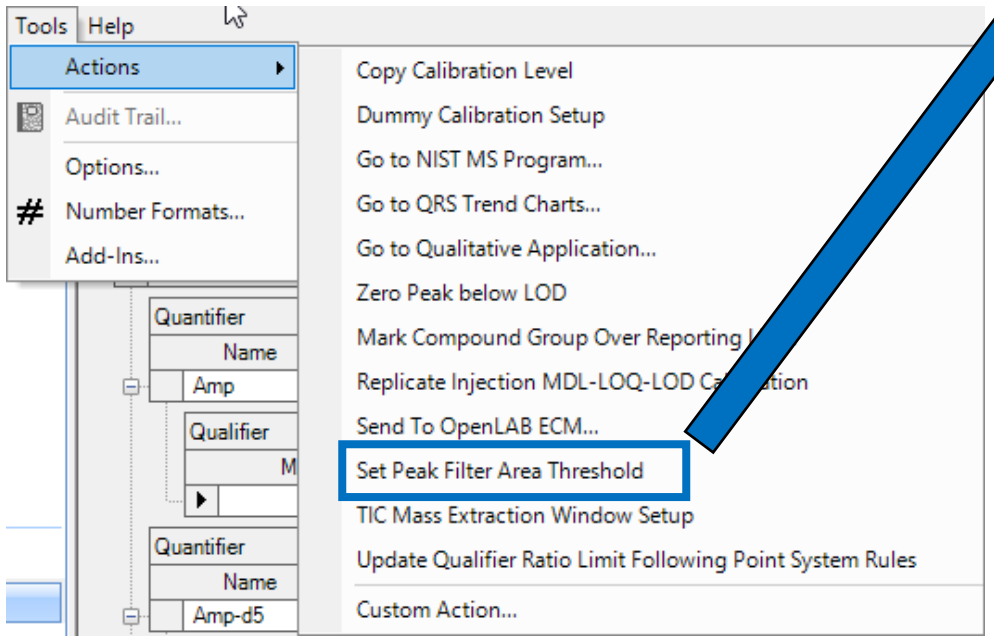
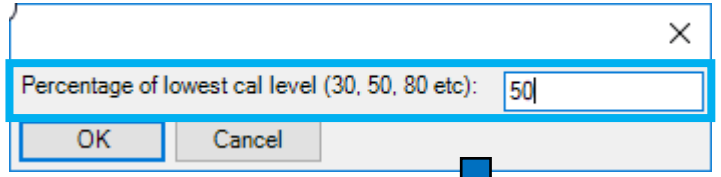
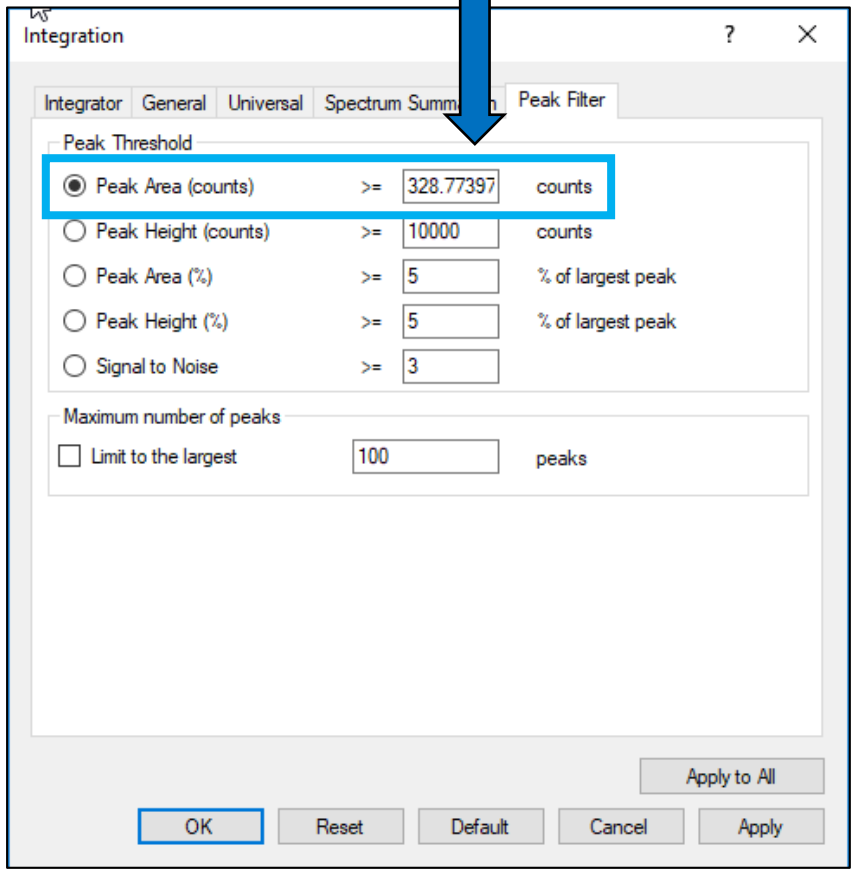
To automate the process, while in the Method Editor view, use the “Set Peak Filter Area Threshold” script to set each compound to a percent of its lowest Calibrator in the batch.

Tip: Establish calibration curve first.







Adjusting Peak Filter Area Thresholds

Example

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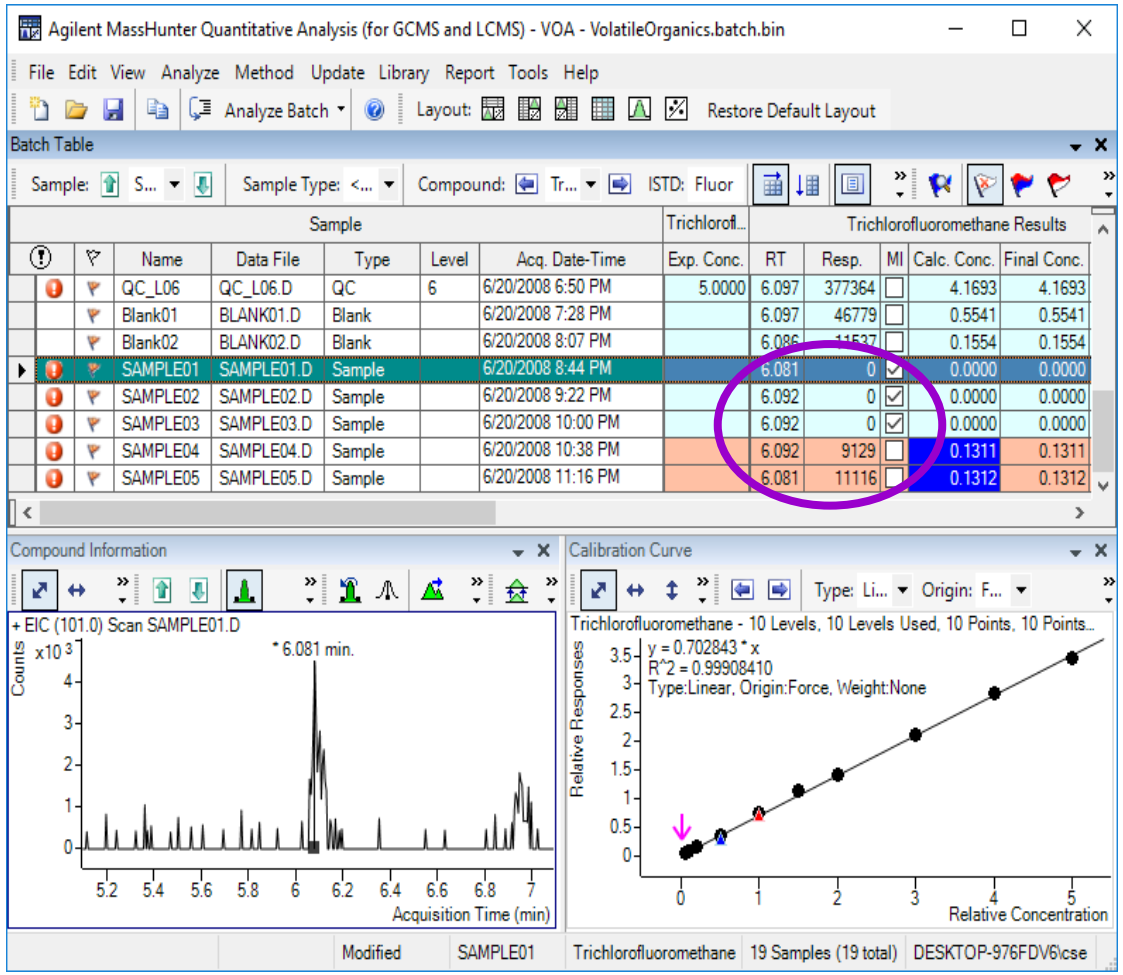

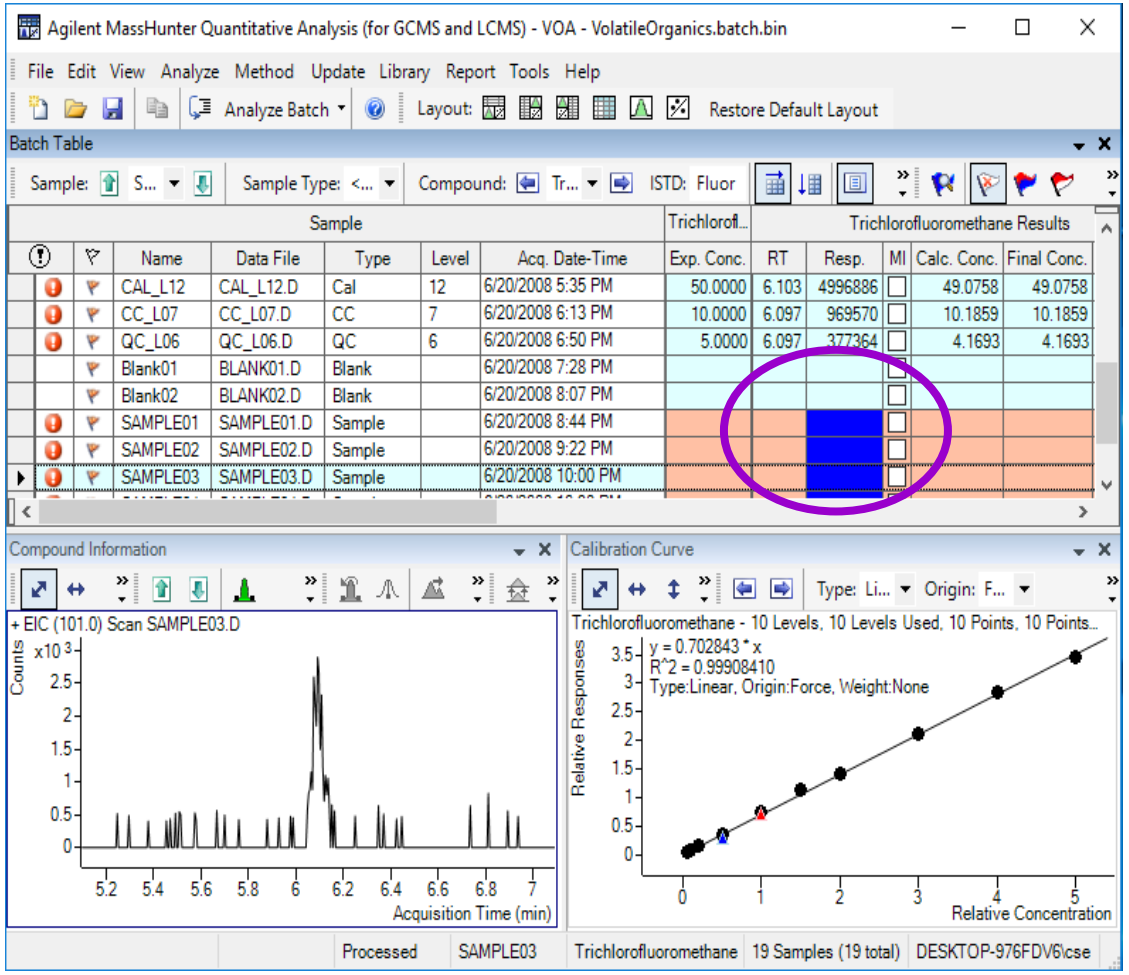

Before **After**

Typical noise or matrix peaks are removed and require no data review or “zero peak” work.

Zero Peak Below LOD

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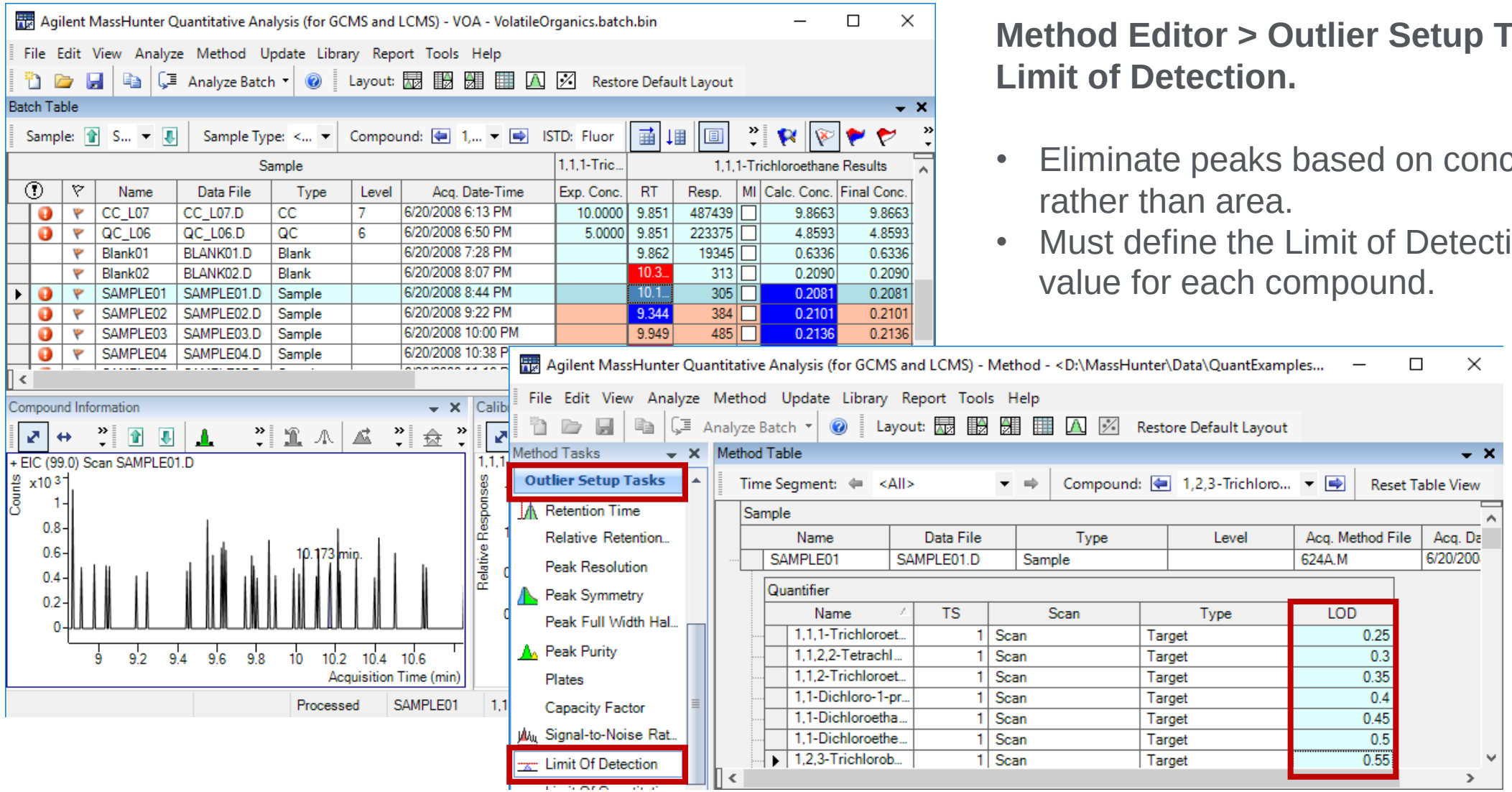
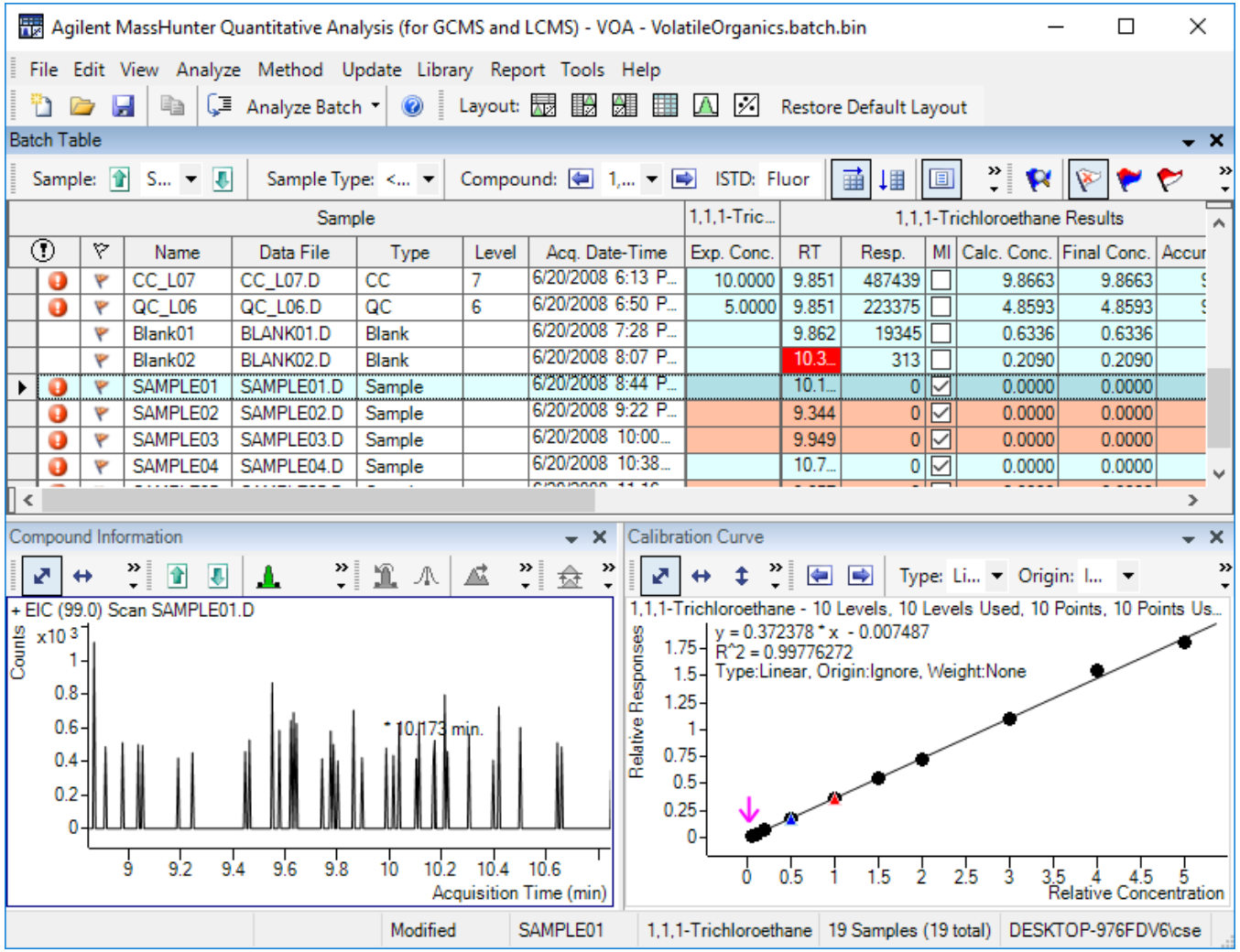


Method Editor > Outlier Setup Tasks > Limit of Detection.

- Eliminate peaks based on concentration rather than area.
- Must define the Limit of Detection outlier value for each compound.

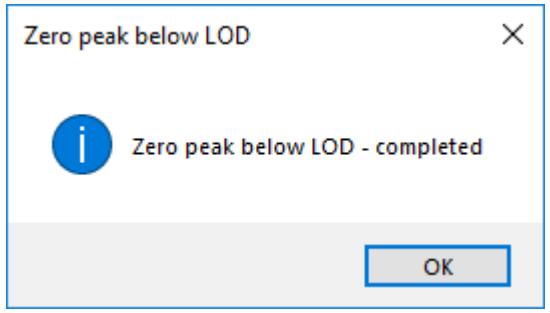
Zero Peak Below LOD



Tools > Actions > Zero Peak Below LOD

Tip: Review calibrators first.

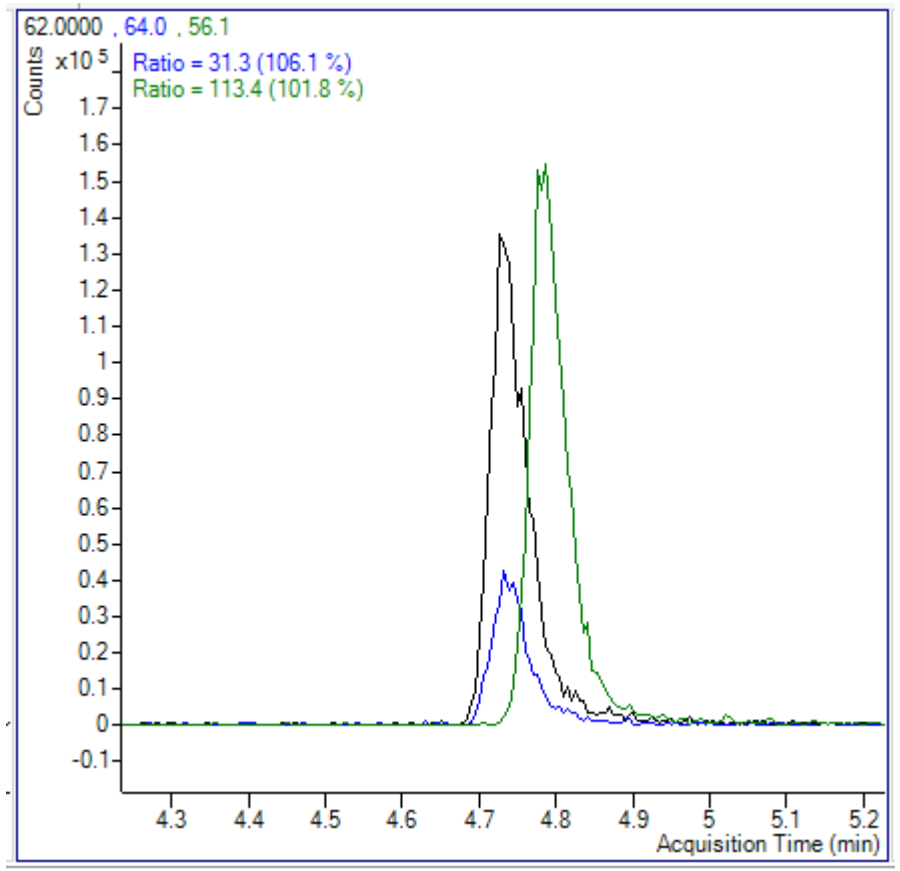
- Accessed from Batch Table View or Method Editor View.
- Zeros compounds if the Calculated Concentration is less than the LOD.

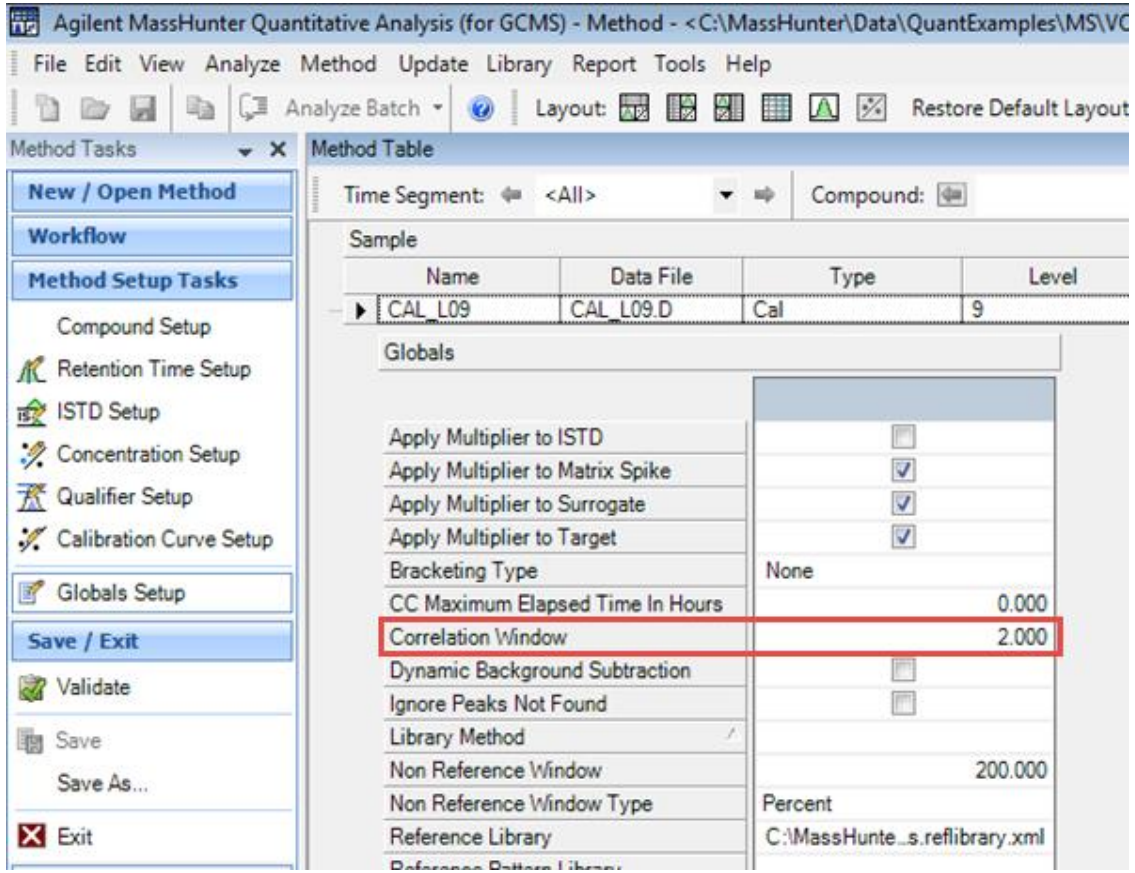


Correlation Window

The retention time difference limit of target ions to one or more qualifiers.

- Defines the maximum allowable variation of multiple extracted ion peak retention times before they are considered a single peak.
- Default time of 2.00 min is rather wide. Typically 0.01 to 0.05 min (0.6 sec to 3.0 sec).





Correlation Window

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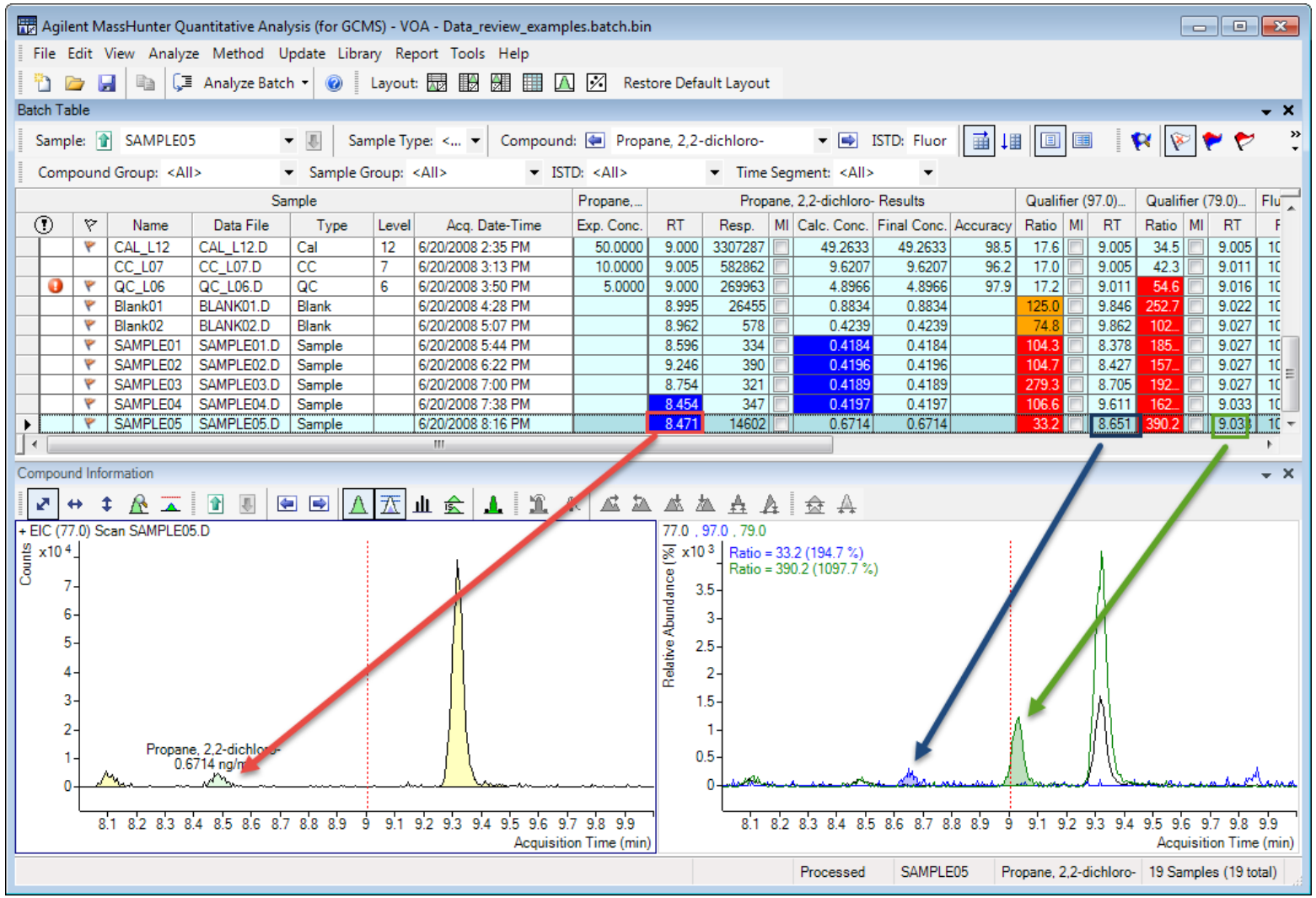
Default value = 2.0 minutes.



Note that the retention times for target and qualifiers are different!

Retention Time Drift

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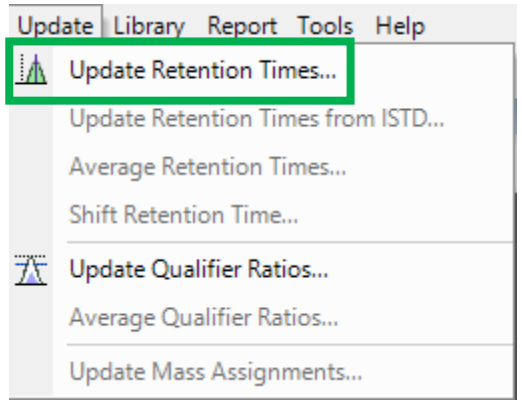
Retention times will Drift

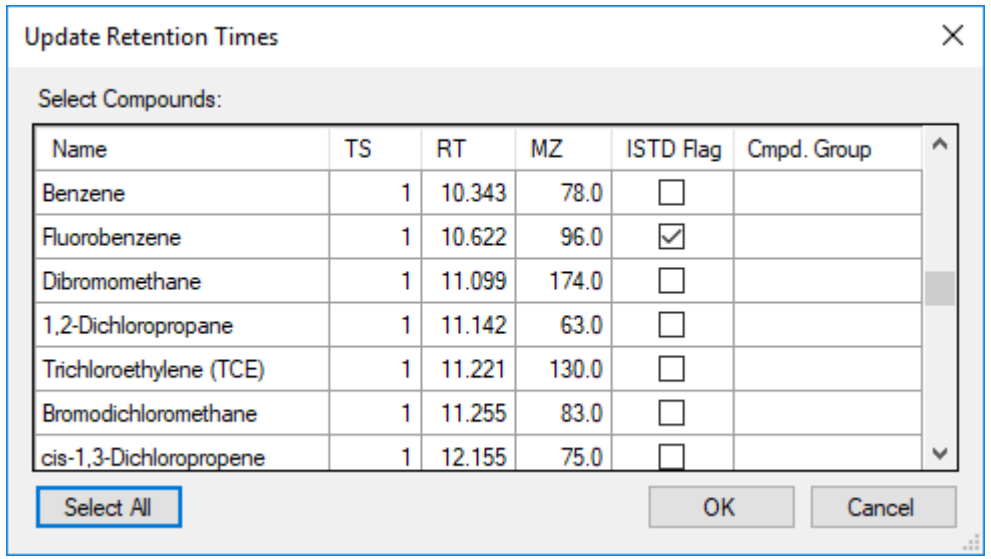
- GC
  - Can use RTL locking to mitigate drift.
  - Use time reference compound in the middle of chromatographic run.
  - Make a single compound standard in the mid range of concentration.
  - Lock the method.
  - Relock the method as necessary.

- LC
  - Utilize a guard column.
  - Sample preparation steps.
  - Change solvents frequently to avoid microbial growth.
  - Clean solvent bottles when changing solvent.

But…retention times will still drift.

Retention Time Drift
Update Retention Times

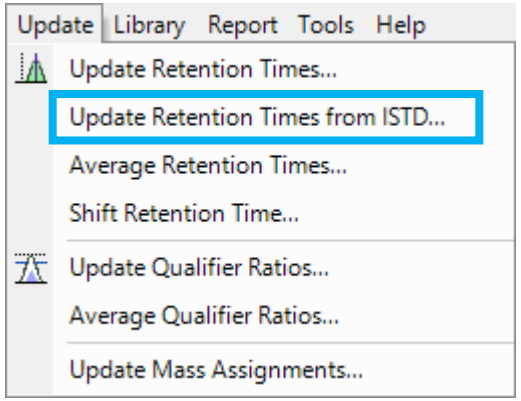


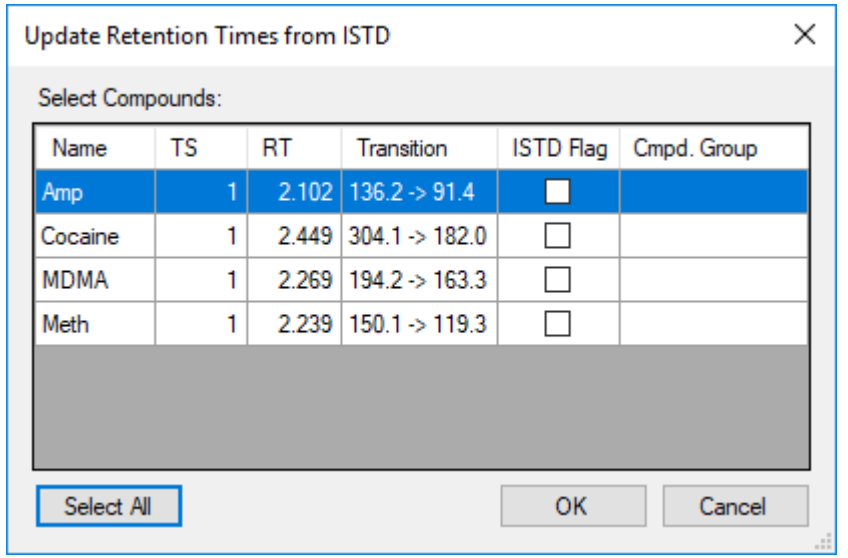


Update Retention Times is available in Batch Table View and Method Editor View.
Caveat - the compound must be found to update it.

Tip: Best updated from a mid range calibrator.

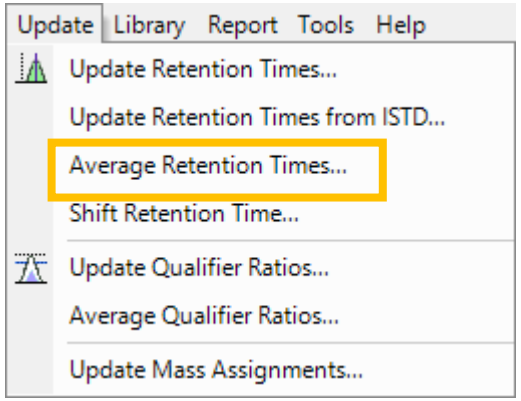
Retention Time Drift
Update Retention Times from ISTD

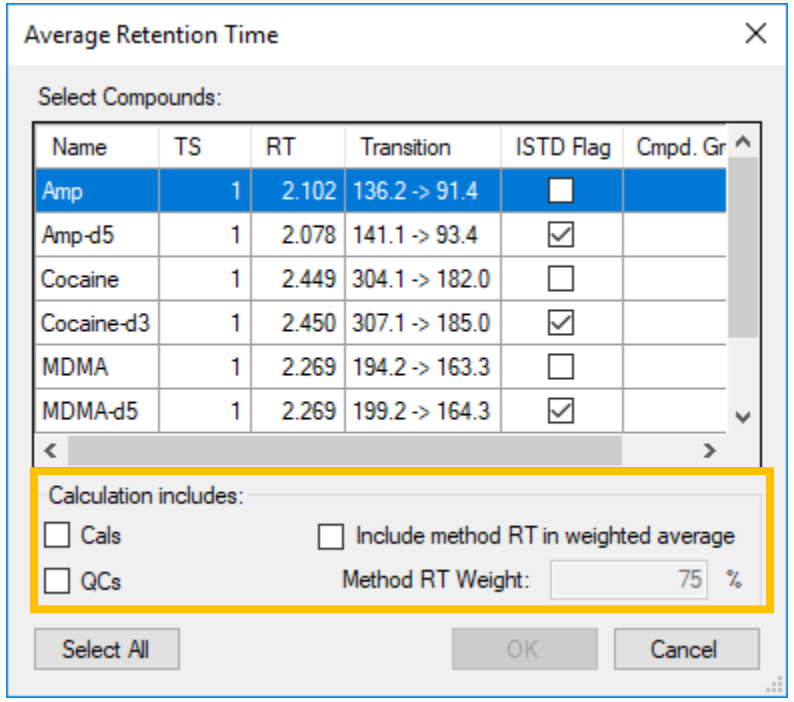




- Update Retention Times from ISTD is a Method Editor feature.
- Particularly useful with isotopically labeled ISTD.
- **Tip: Remember Method Editor see one and only one sample—choose a calibrator.**

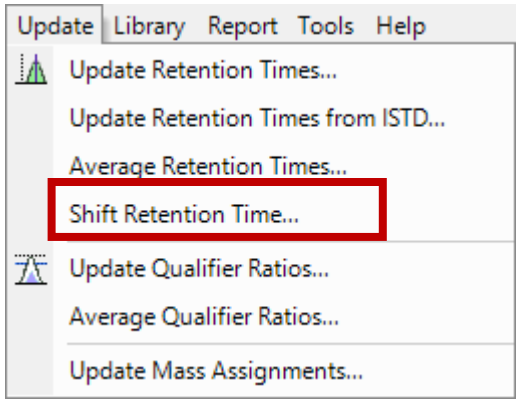
Retention Time Drift
Average Retention Times

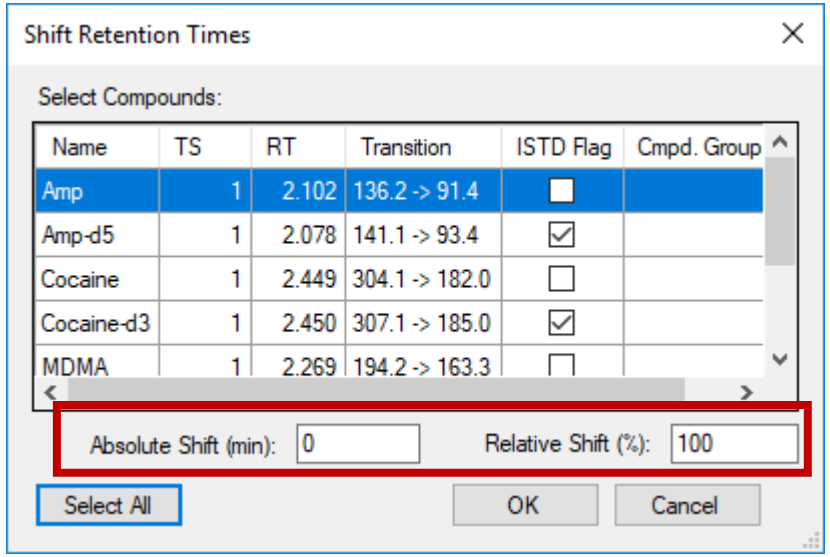




- Average Retention Times is a Method Editor feature.
- Allows choice of Cals or QCs or both.
- Includes a weighted average.

Retention Time Drift
Shift Retention Time





- Shift Retention Time is a Method Editor feature.
- Allows an Absolute Shift in minutes or a Relative Shift in percentage.
- Would be most applicable when changing columns.

Qualifier Ion Ratios

Based on the sample currently loaded in Method Editor.

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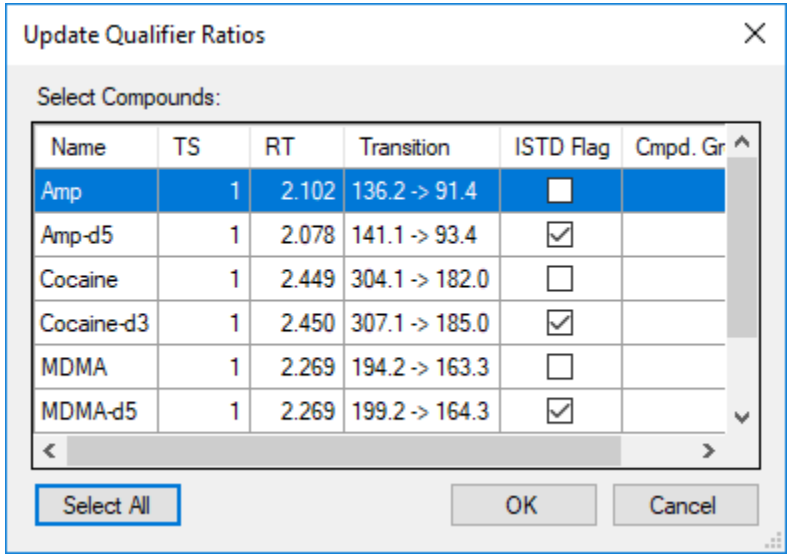
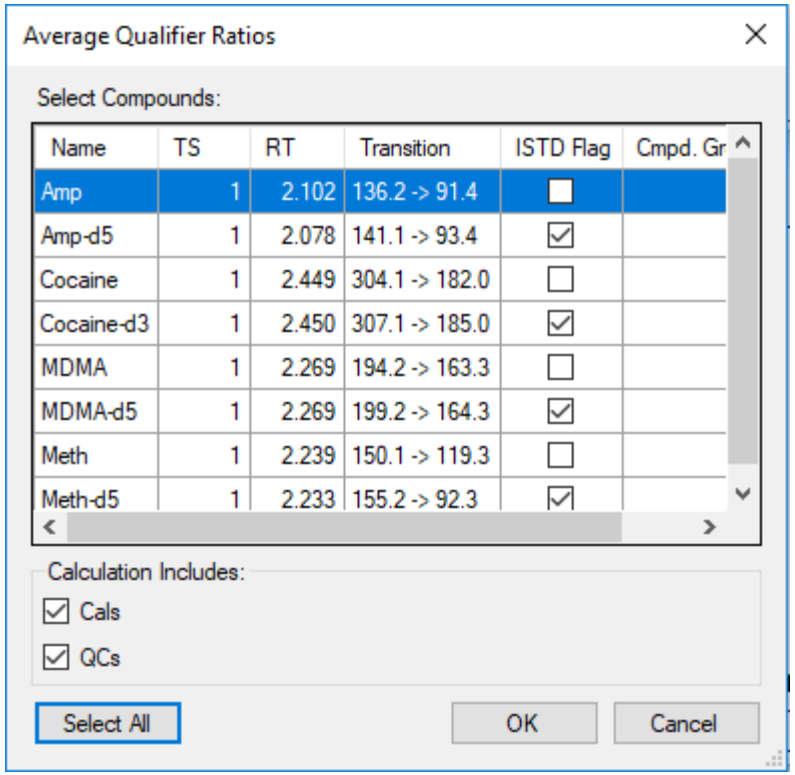
Tip: Choose a mid range calibrator before entering the Method Editor.

Update Qualifier Ratios



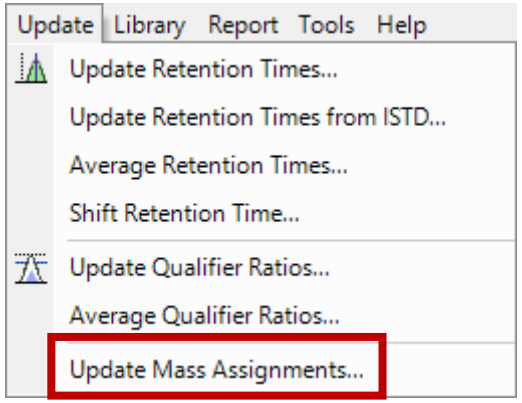
Or

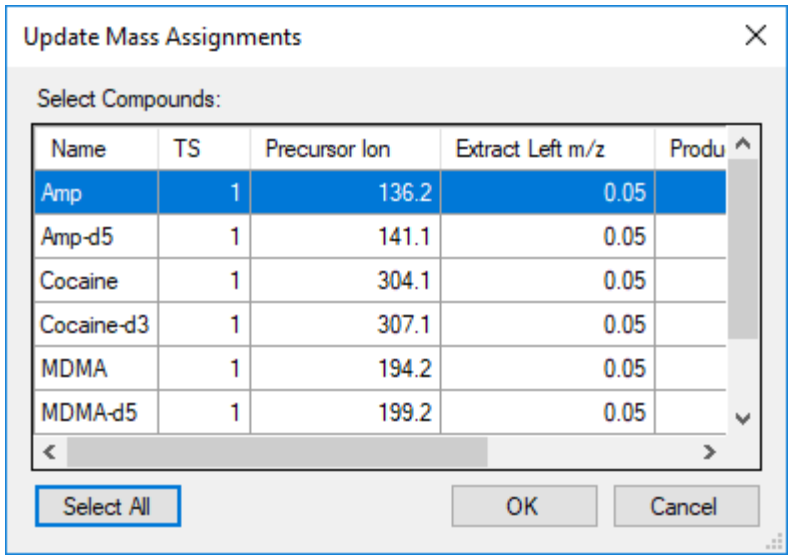
Average Qualifier Ratios



Can use Cals and/or QCs.

Update Mass Assignments

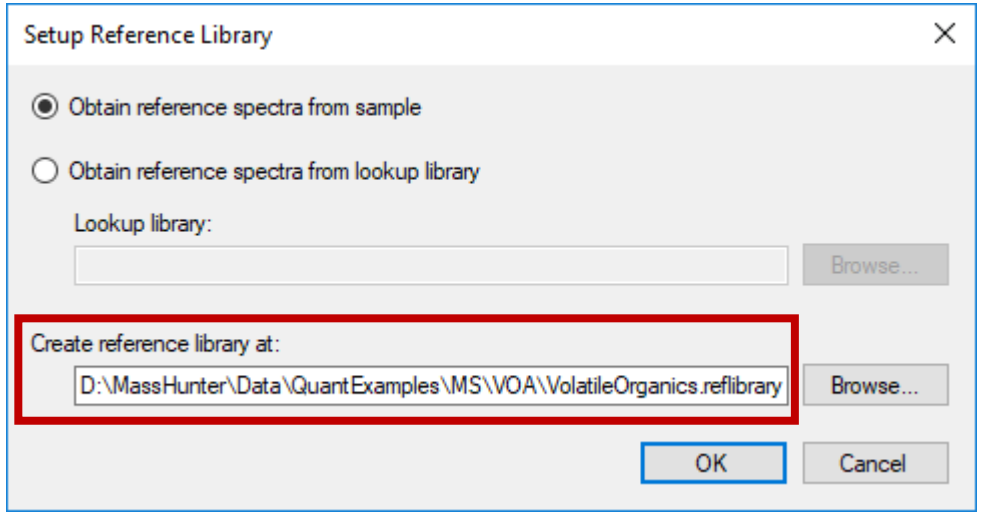


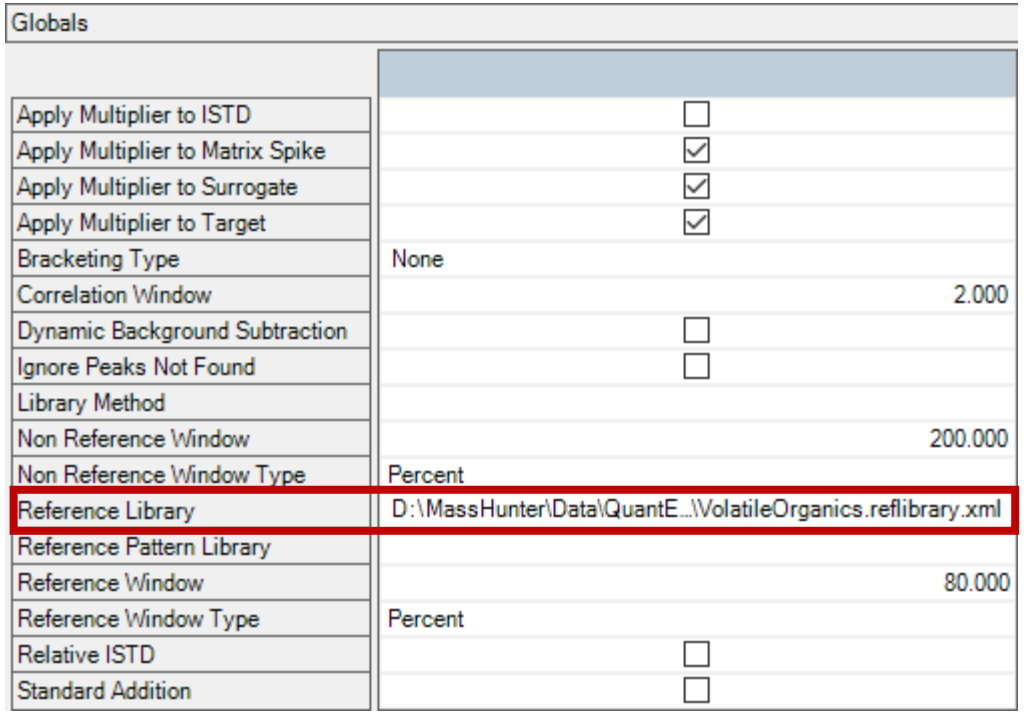


- Update Mass Assignments is a Method Editor feature.
- It is based on currently selected sample in Batch Table.
- Useful with high resolution data (TOF and QTOF).

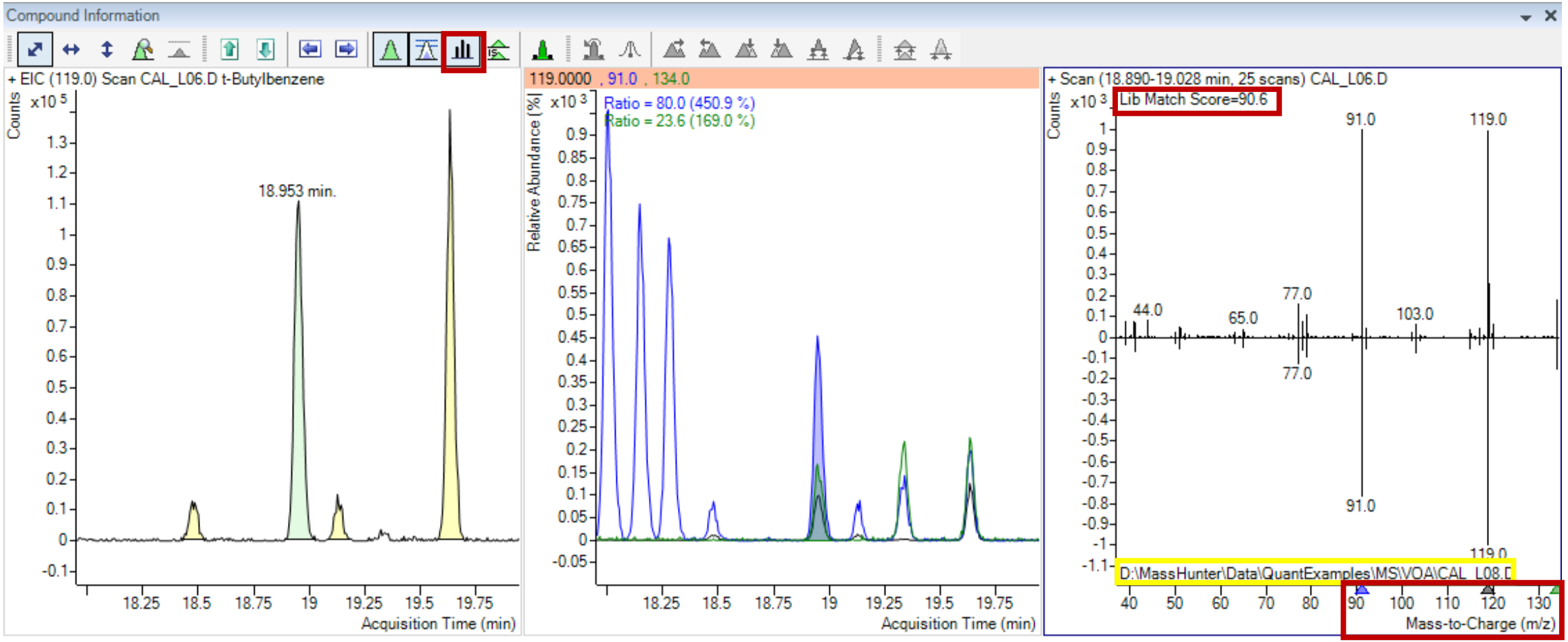
Reference Library
Globals Settings Option

- Activated from **Method > Edit > Library > Setup Reference Library**
- Reference Library may be obtained from a sample, preferably a calibrator, or from a small user generated library or a small subset library.
- Reference Library name is auto populated in Globals Settings.





Reference Library
Globals Settings Option

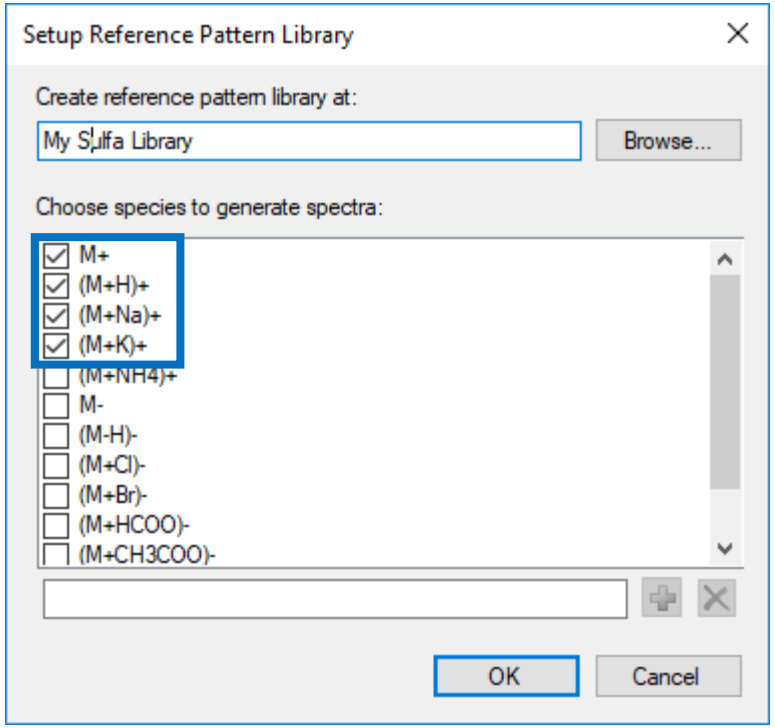


Appears in Compound Information window.
Customizable in **right click > Properties > Compound Information (2) tab.**

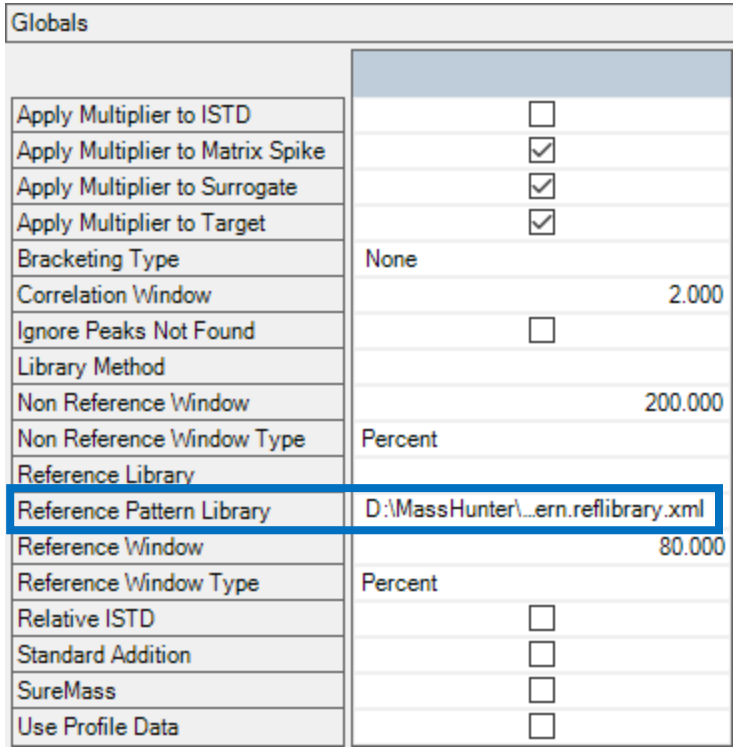
Reference Pattern Library

High Resolution Data

- Activated from **Method > Edit > Library > Setup Reference Pattern Library.**
- Can be obtained from a calibrator or from another library.
- Method must contain molecular formula.
- Reference Pattern Library name is populated in Globals.

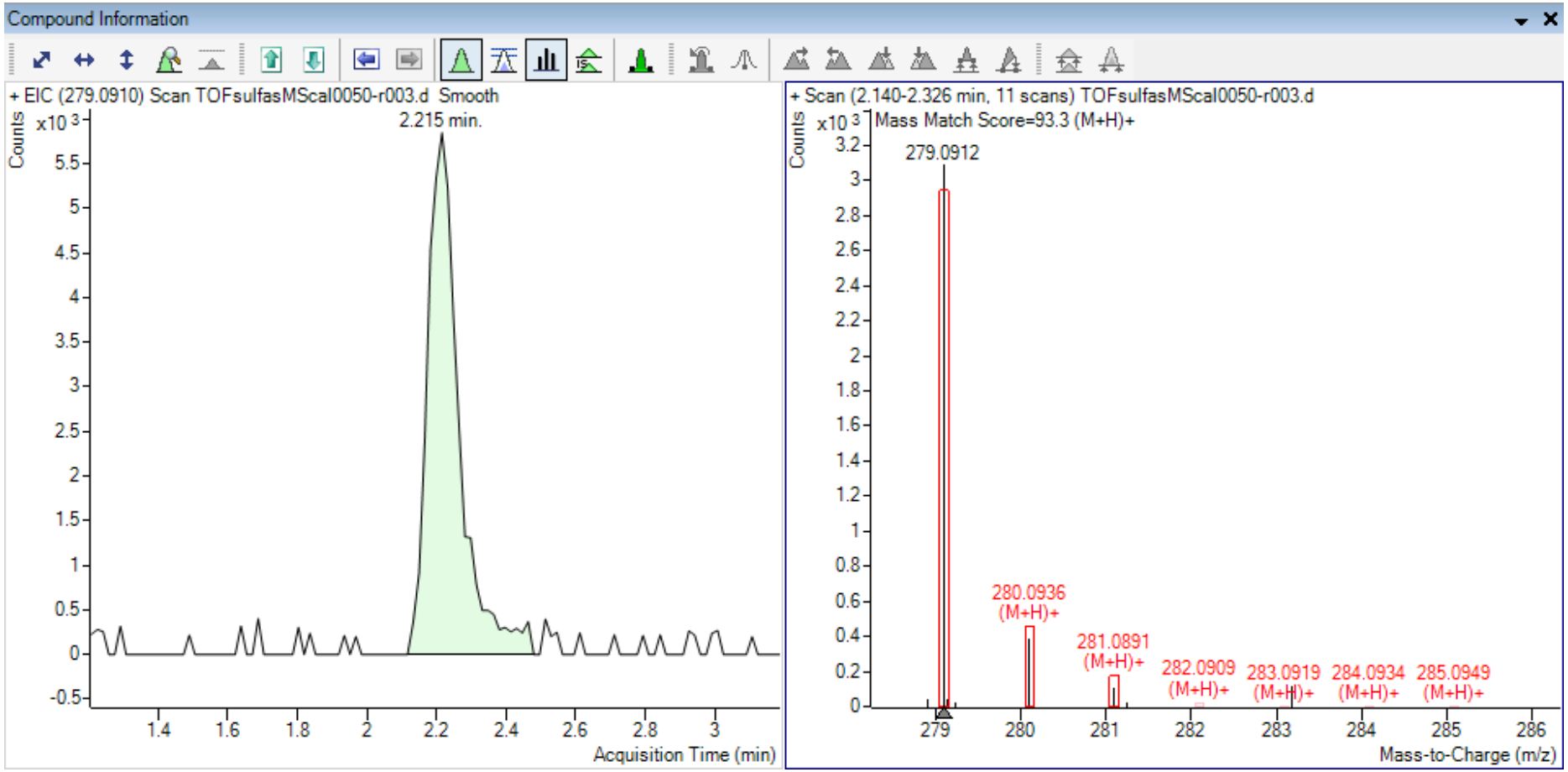


Select the adducts.



Reference Pattern Library
High Resolution Data

- Right click **Properties > Compound Information (2) > Reference pattern spectrum**
- Isotopic abundance and pattern appears in Spectrum window.



Demo time

Data Review Check List

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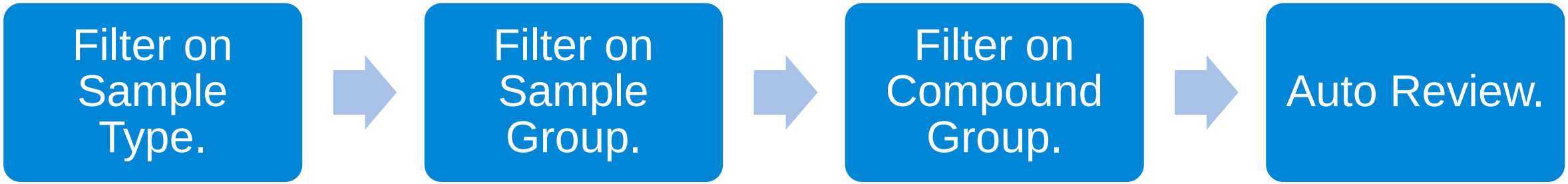
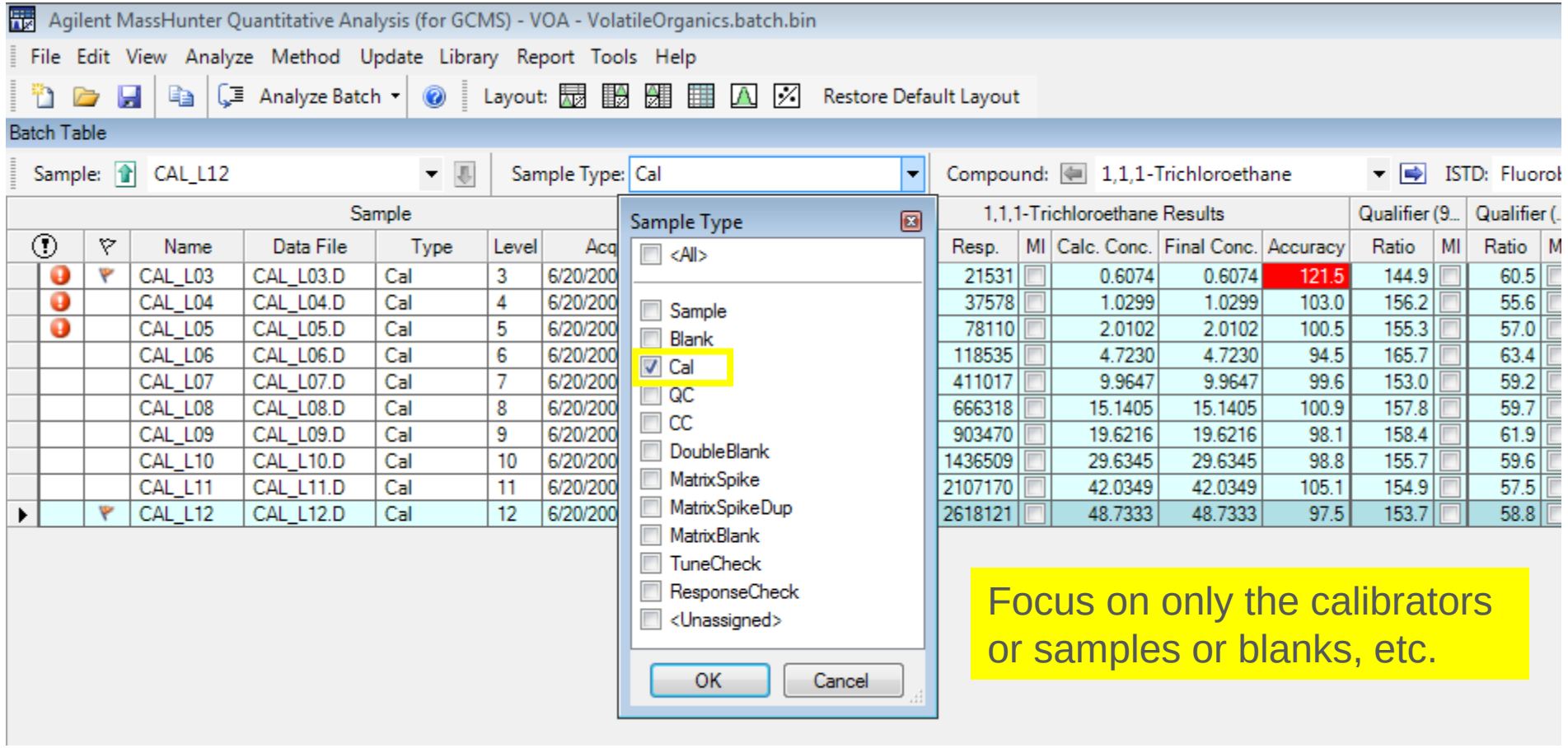


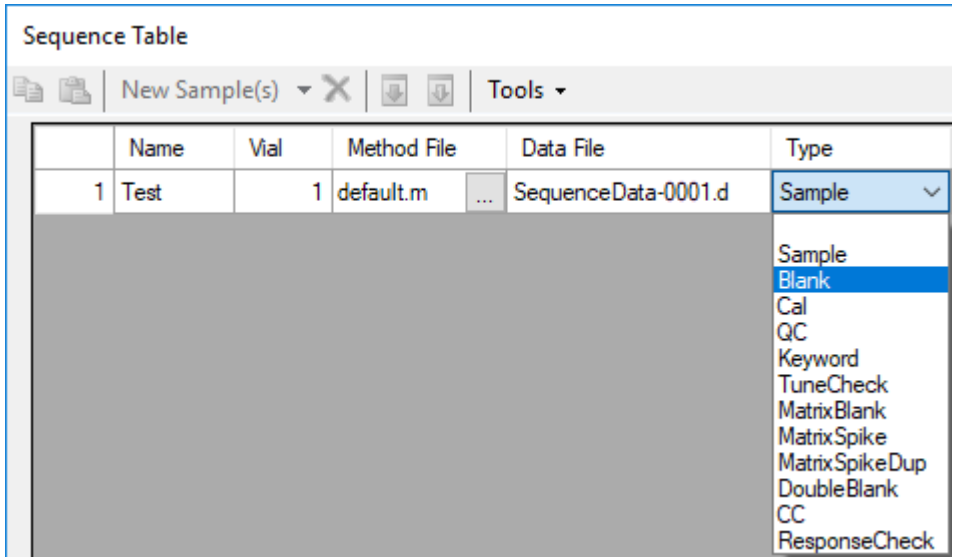
Data Review
Filter on Sample Type

Agilent CrossLab
From Insight to Outcome

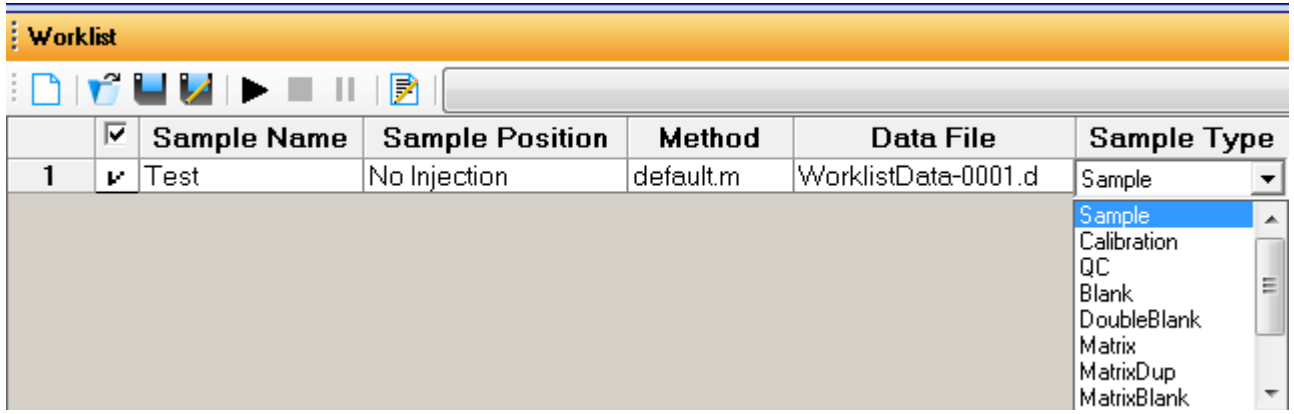
Review Data in stages (Calibrators, then QC, then Samples).



Data Review
Sample Type



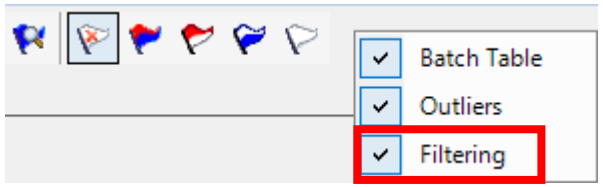
- The Type or Sample Type is a parameter available in the sequence or worklist.
- Should be specified when data is acquired, but if necessary it can be entered in the Batch Table.
- In Sequence Table in GCMS software.
- In Worklist in LC MassHunter software.

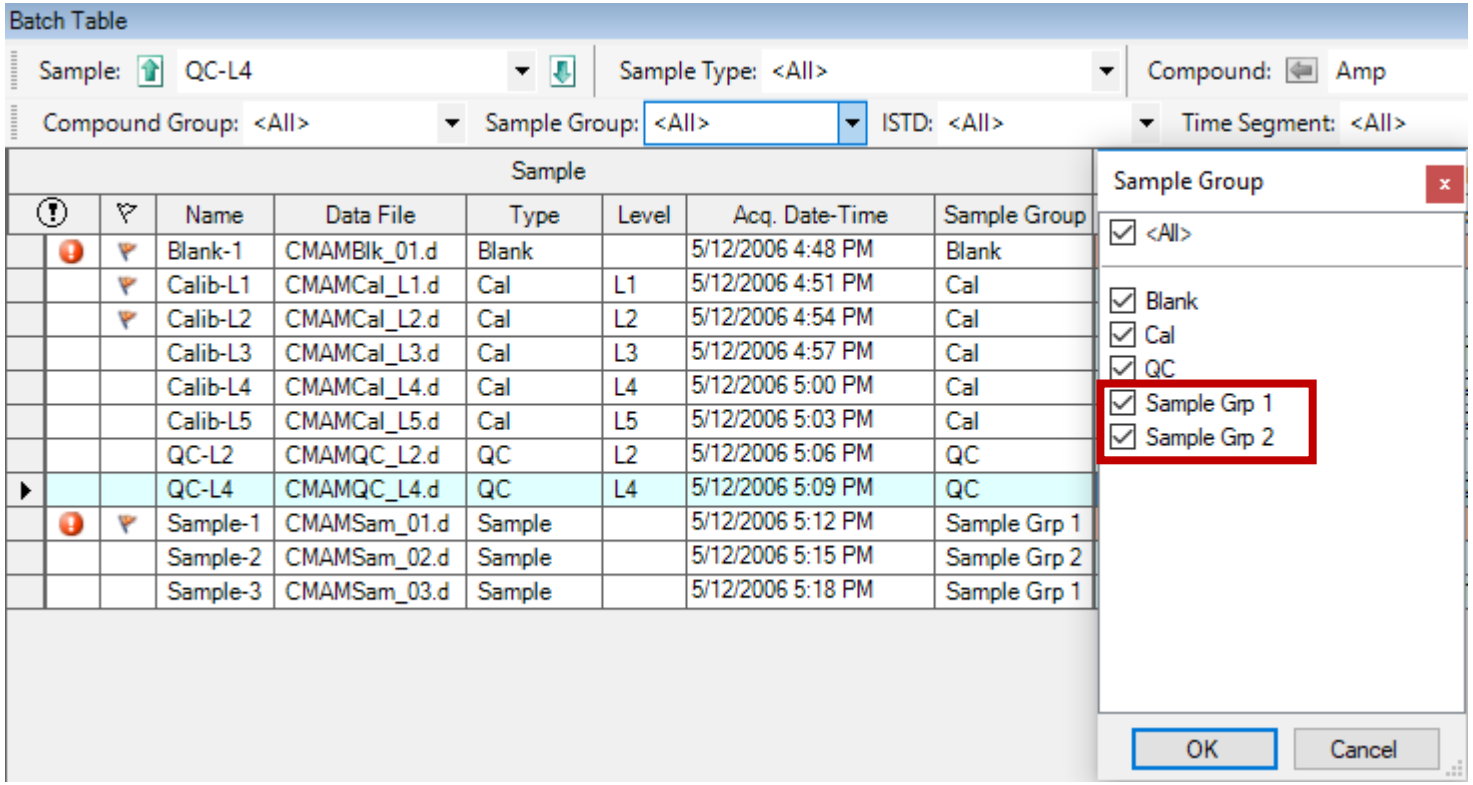


Data Review
Filter on Sample Group

Agilent CrossLab
From Insight to Outcome

Sample Group is activated by right clicking on the toolbar.





- This is helpful with large batches that contain several sample types.
- Sample Group is a column that can be added to the Worklist or Sequence Table.

Data Review
Filter on Compound Group

Agilent CrossLab
From Insight to Outcome

Compound groups are assigned in the Method Editor.

Quantifier

Name	TS	Scan	Type	MZ	RT	Ion Polarity	Criteria	Cmpd. Group	Compound Math
4,4'-Dibromooct...	1	Scan	ISTD	456.0	12.808	Positive	Close RT		
Aldrin	1	Scan	Target	263.0	19.671	Positive	Close RT		
Azinphos-ethyl	1	Scan	Target	132.0	31.018	Positive	Close RT		
Azinphos-methyl	1	Scan	Target	160.0	30.082	Positive	Close RT		
BHC alpha isom...	1	Scan	Target	181.0	13.185	Positive	Close RT	BHC	
BHC beta isomer	1	Scan	Target	219.0	14.327	Positive	Close RT	BHC	
BHC delta isomer	1	Scan	Target	181.0	15.693	Positive	Close RT	BHC	
BHC Total	1	Scan	Target	100.0	15.000	Positive	Close RT	BHC	
Carbophenothion	1	Scan	Target	157.0	27.267	Positive	Close RT		
Chlorpyrifos	1	Scan	Target	197.0	20.355	Positive	Close RT		

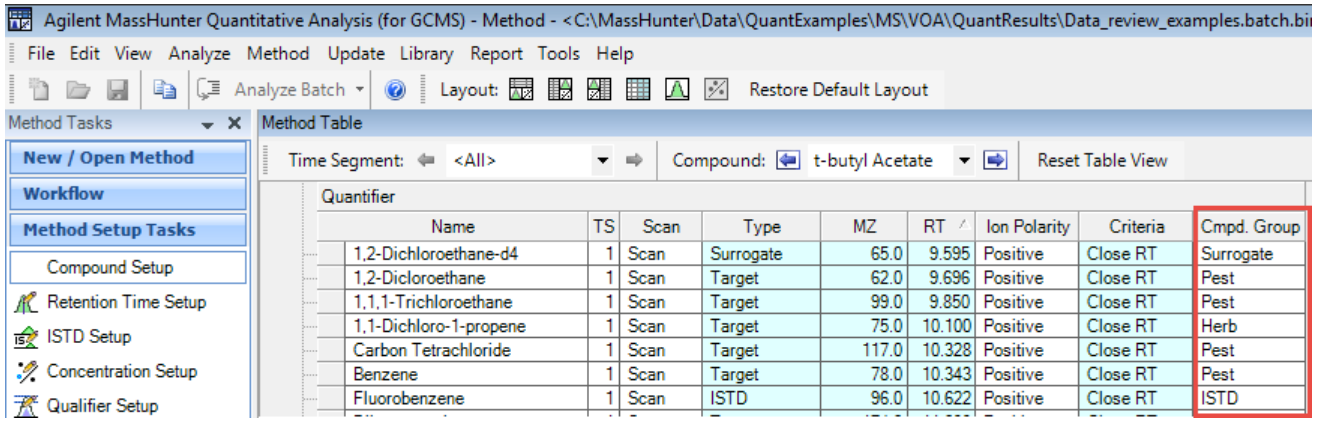
Response Sum
Response Average
Concentration Sum
Concentration Average
TPH Subtraction

- Compound groups are useful for parent compound and metabolites.
- Reviewing Aroclor congeners by group (PCB).

Data Review

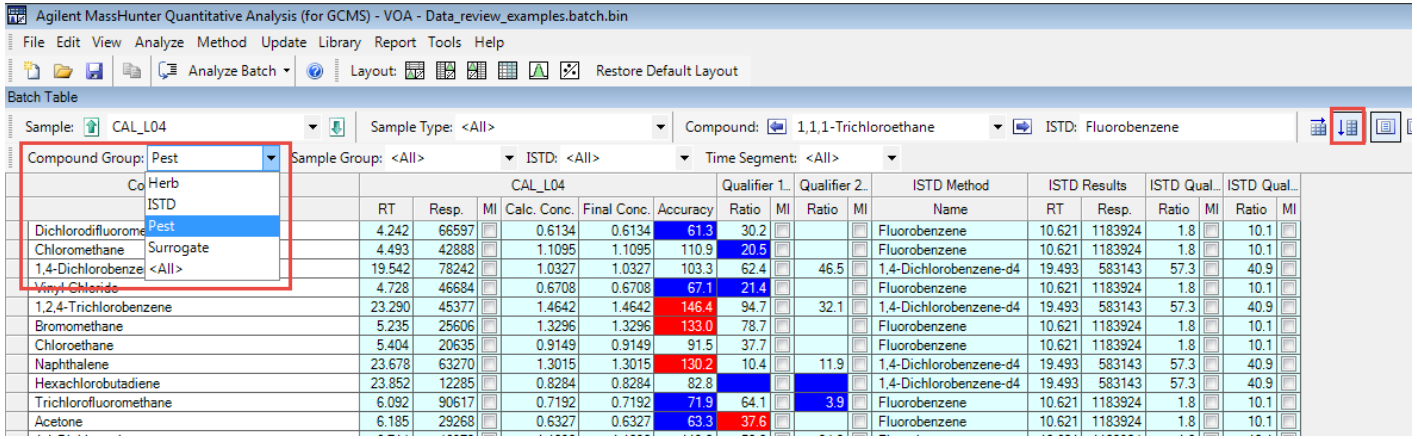
Filter on Compound Group

Compounds may be assigned to more than one group by separating the group names using commas.



Name	TS	Scan	Type	MZ	RT	Ion Polarity	Criteria	Cmpd. Group
1,2-Dichloroethane-d4	1	Scan	Surrogate	65.0	9.595	Positive	Close RT	Surrogate
1,2-Dicloroethane	1	Scan	Target	62.0	9.696	Positive	Close RT	Pest
1,1,1-Trichloroethane	1	Scan	Target	99.0	9.850	Positive	Close RT	Pest
1,1-Dichloro-1-propene	1	Scan	Target	75.0	10.100	Positive	Close RT	Herb
Carbon Tetrachloride	1	Scan	Target	117.0	10.328	Positive	Close RT	Pest
Benzene	1	Scan	Target	78.0	10.343	Positive	Close RT	Pest
Fluorobenzene	1	Scan	ISTD	96.0	10.622	Positive	Close RT	ISTD

Review Compounds by Group (using Compound Table View).

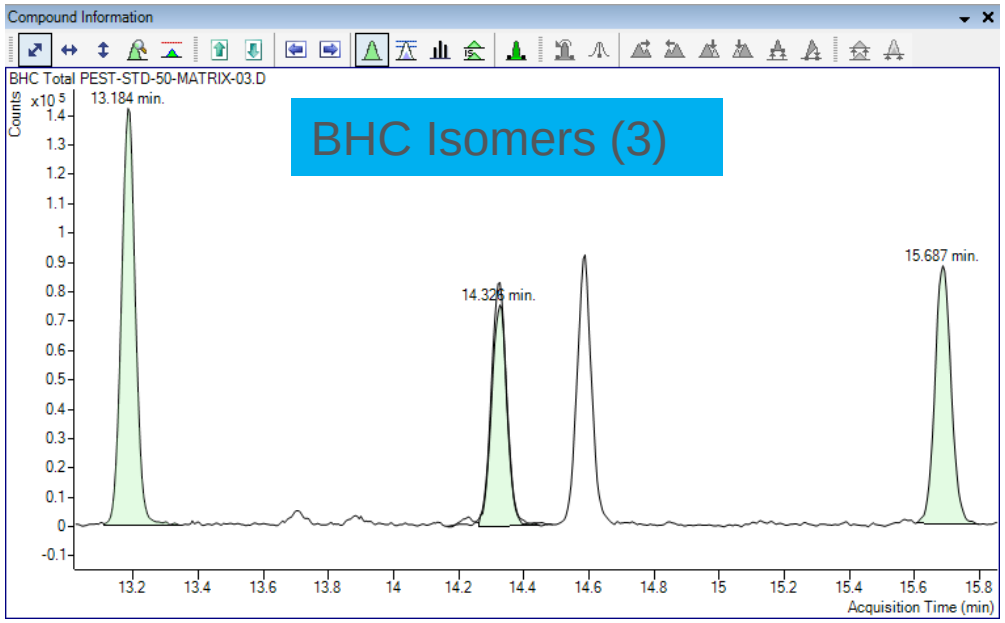


	CAL_L04						Qualifier 1..		Qualifier 2..		ISTD Method	ISTD Results		ISTD Qual..		ISTD Qual..	
	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	Name	RT	Resp.	Ratio	MI	Ratio	MI
Dichlorodifluorome	4.242	66597		0.6134	0.6134	61.3	30.2				Fluorobenzene	10.621	1183924	1.8		10.1	
Chloromethane	4.493	42888		1.1095	1.1095	110.9	20.5				Fluorobenzene	10.621	1183924	1.8		10.1	
1,4-Dichlorobenze	19.542	78242		1.0327	1.0327	103.3	62.4		46.5		1,4-Dichlorobenzene-d4	19.493	583143	57.3		40.9	
Vinyl Chloride	4.728	46684		0.6708	0.6708	67.1	21.4				Fluorobenzene	10.621	1183924	1.8		10.1	
1,2,4-Trichlorobenzene	23.290	45377		1.4642	1.4642	146.4	94.7		32.1		1,4-Dichlorobenzene-d4	19.493	583143	57.3		40.9	
Bromomethane	5.235	25606		1.3296	1.3296	133.0	78.7				Fluorobenzene	10.621	1183924	1.8		10.1	
Chloroethane	5.404	20635		0.9149	0.9149	91.5	37.7				Fluorobenzene	10.621	1183924	1.8		10.1	
Naphthalene	23.678	63270		1.3015	1.3015	130.2	10.4		11.9		1,4-Dichlorobenzene-d4	19.493	583143	57.3		40.9	
Hexachlorobutadiene	23.852	12285		0.8284	0.8284	82.8					1,4-Dichlorobenzene-d4	19.493	583143	57.3		40.9	
Trichlorofluoromethane	6.092	90617		0.7192	0.7192	71.9	64.1		3.9		Fluorobenzene	10.621	1183924	1.8		10.1	
Acetone	6.185	29268		0.6327	0.6327	63.3	37.6				Fluorobenzene	10.621	1183924	1.8		10.1	

Data Review

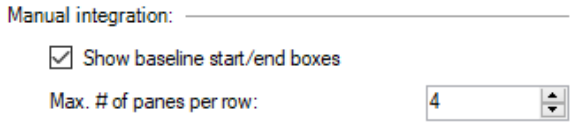
Compound Information

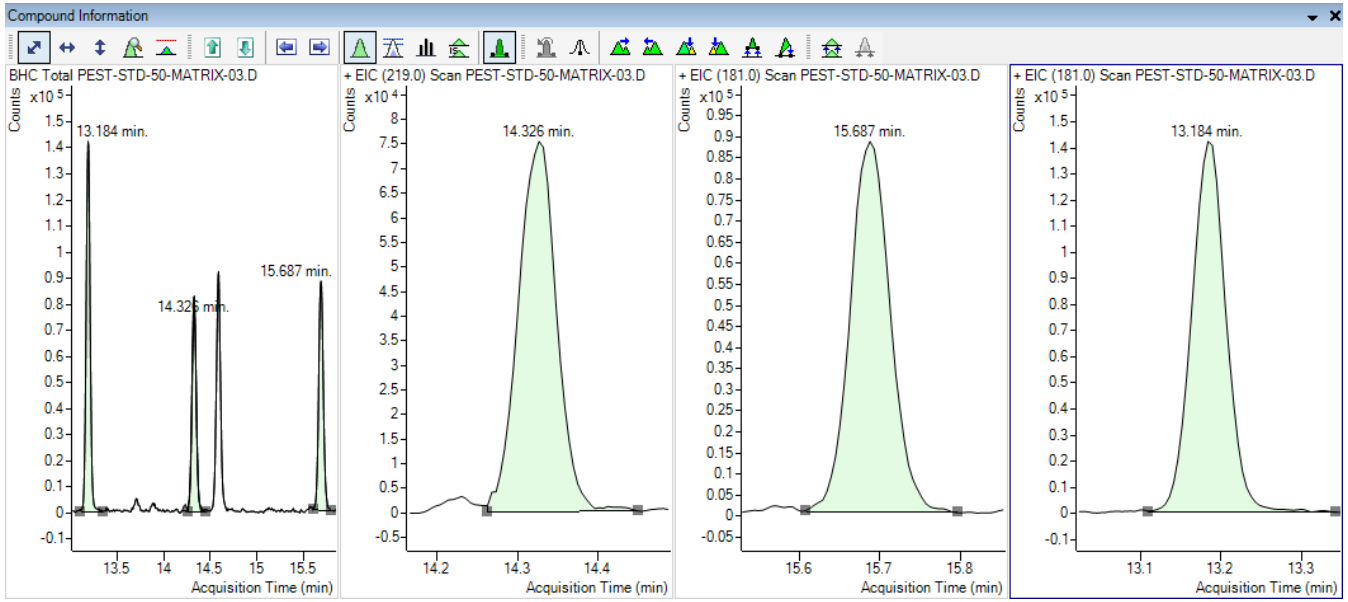
Compound groups are shown in the Compound information window.



BHC Isomers (3)

Tip: Right click Properties > Compound Information (2) Manual integration > Max # panes per row.

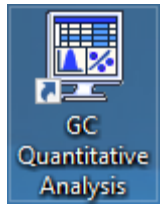


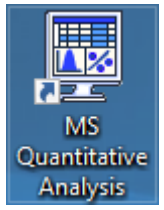


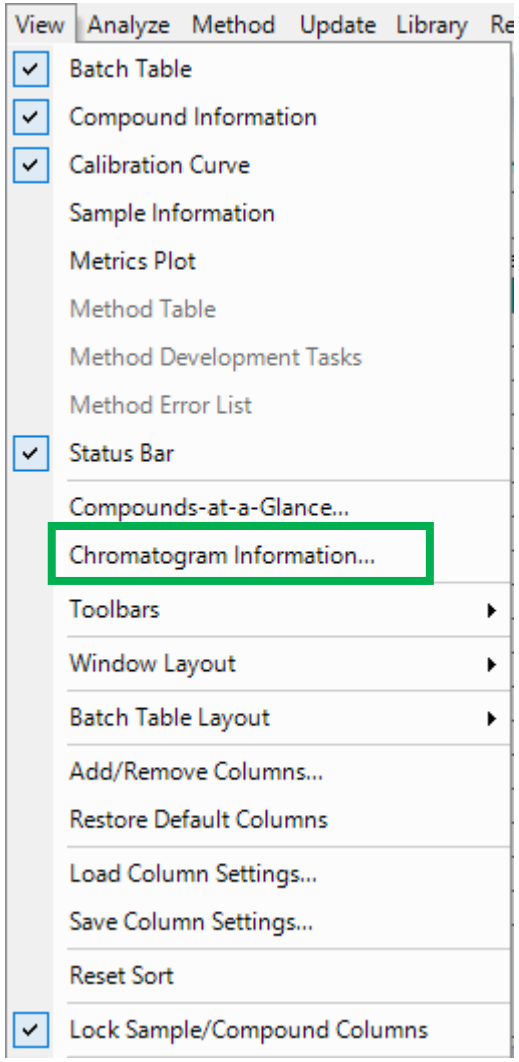
Manual Integration turned on.

Data Review
Chromatogram Information

- Accessed from **View > Chromatogram Information**.
- Useful to compare multiple chromatograms.
- Useful to compare patterns.
- Create Compounds GC Data only → Method Editor.
- Available for GC and MS Quantitative Analysis only.







Data Review

Chromatogram Information

Agilent CrossLab
From Insight to Outcome

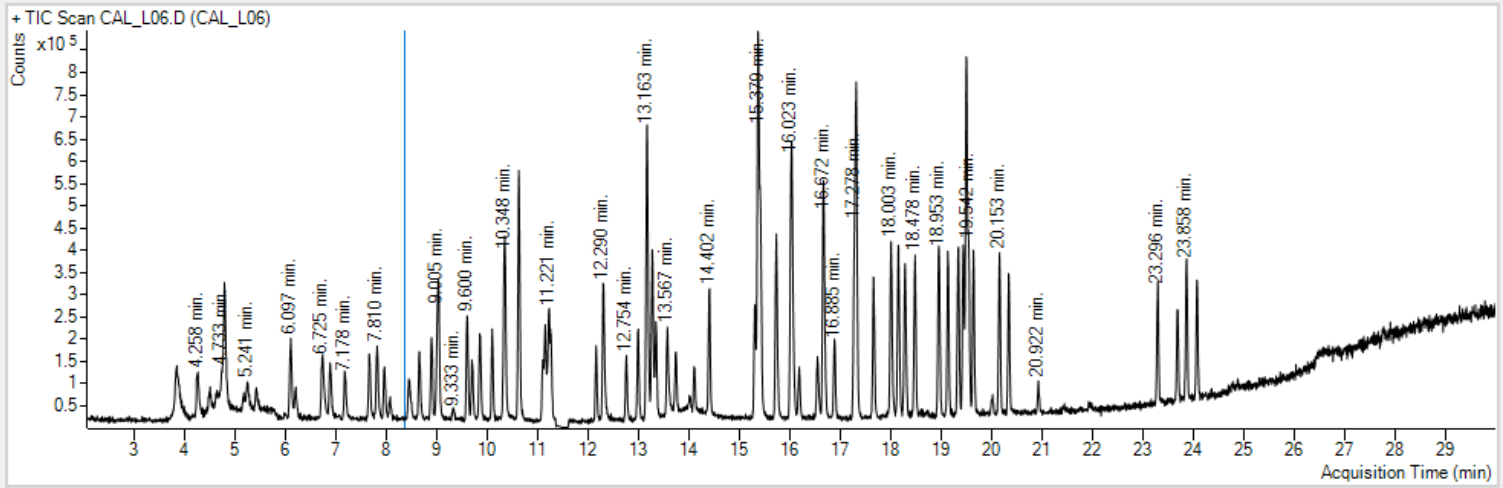
Chromatogram Information

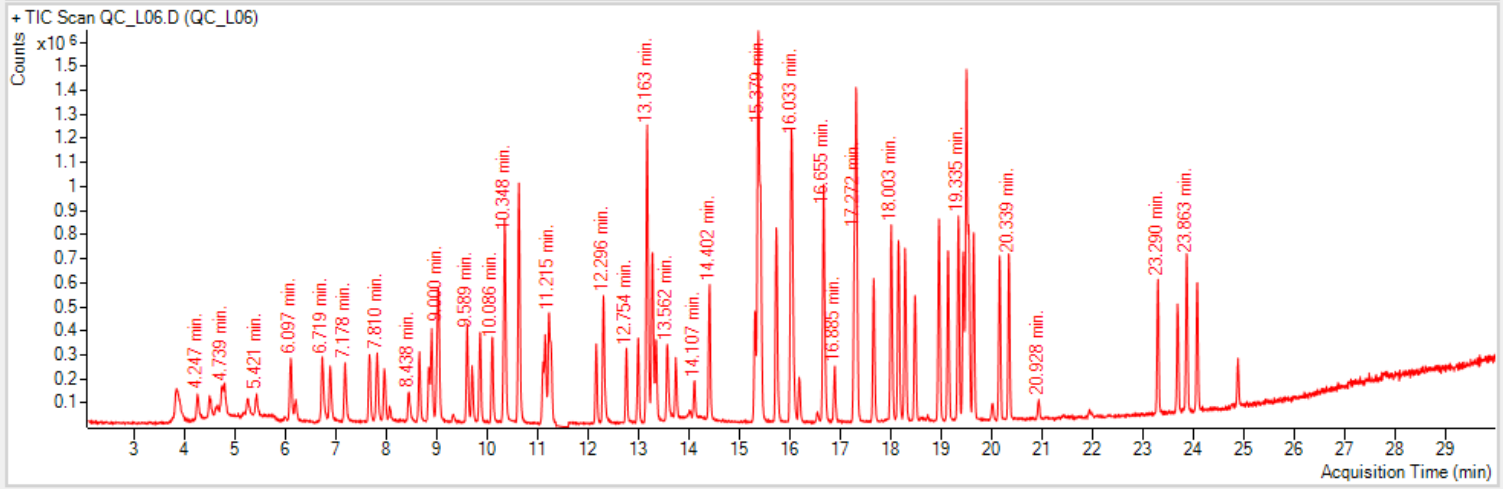
File Edit View

Create Compound | Max # of panes: 2

	Name	Data File	Signal	Color	Anchor
☐	CAL_L03	CAL_L03.D	TIC Scan		☐
☐	CAL_L04	CAL_L04.D	TIC Scan		☐
☐	CAL_L05	CAL_L05.D	TIC Scan		☐
☑	CAL_L06	CAL_L06.D	TIC Scan		☐
☐	CAL_L07	CAL_L07.D	TIC Scan		☐
☐	CAL_L08	CAL_L08.D	TIC Scan		☐
☐	CAL_L09	CAL_L09.D	TIC Scan		☐
☐	CAL_L10	CAL_L10.D	TIC Scan		☐
☐	CAL_L11	CAL_L11.D	TIC Scan		☐
☐	CAL_L12	CAL_L12.D	TIC Scan		☐
☐	CC_L07	CC_L07.D	TIC Scan		☐
☑	QC_L06	QC_L06.D	TIC Scan		☐
☐	Blank01	BLANK01.D	TIC Scan		☐
☐	Blank02	BLANK02.D	TIC Scan		☐
☐	SAMPLE01	SAMPLE01.D	TIC Scan		☐
☐	SAMPLE02	SAMPLE02.D	TIC Scan		☐
☐	SAMPLE03	SAMPLE03.D	TIC Scan		☐
☐	SAMPLE04	SAMPLE04.D	TIC Scan		☐
☐	SAMPLE05	SAMPLE05.D	TIC Scan		☐

Head to head view.
Can anchor a chromatogram.





Data Review

Chromatogram Information

Agilent CrossLab
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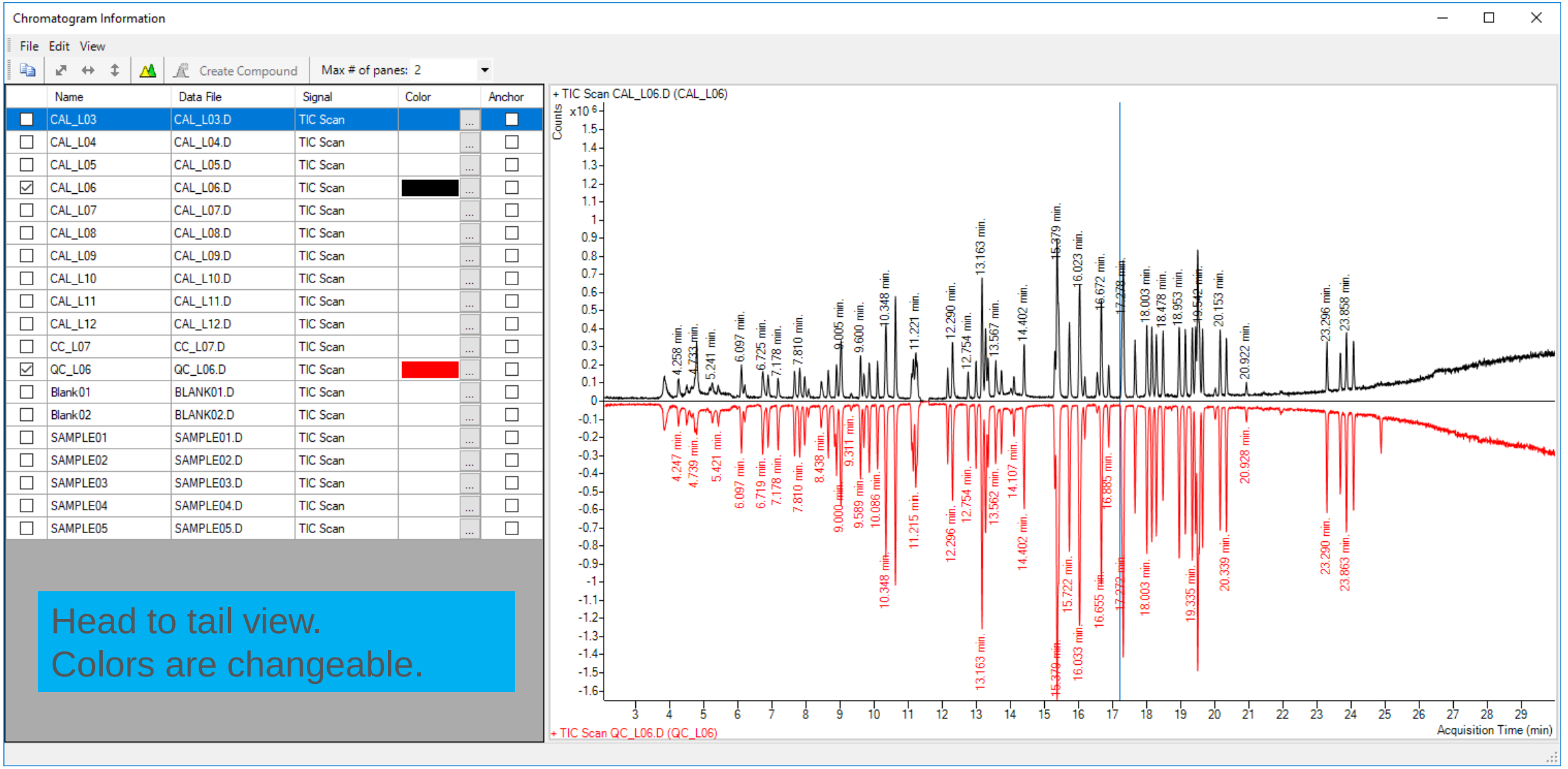


Data Review

Chromatogram Information

Agilent CrossLab
From Insight to Outcome

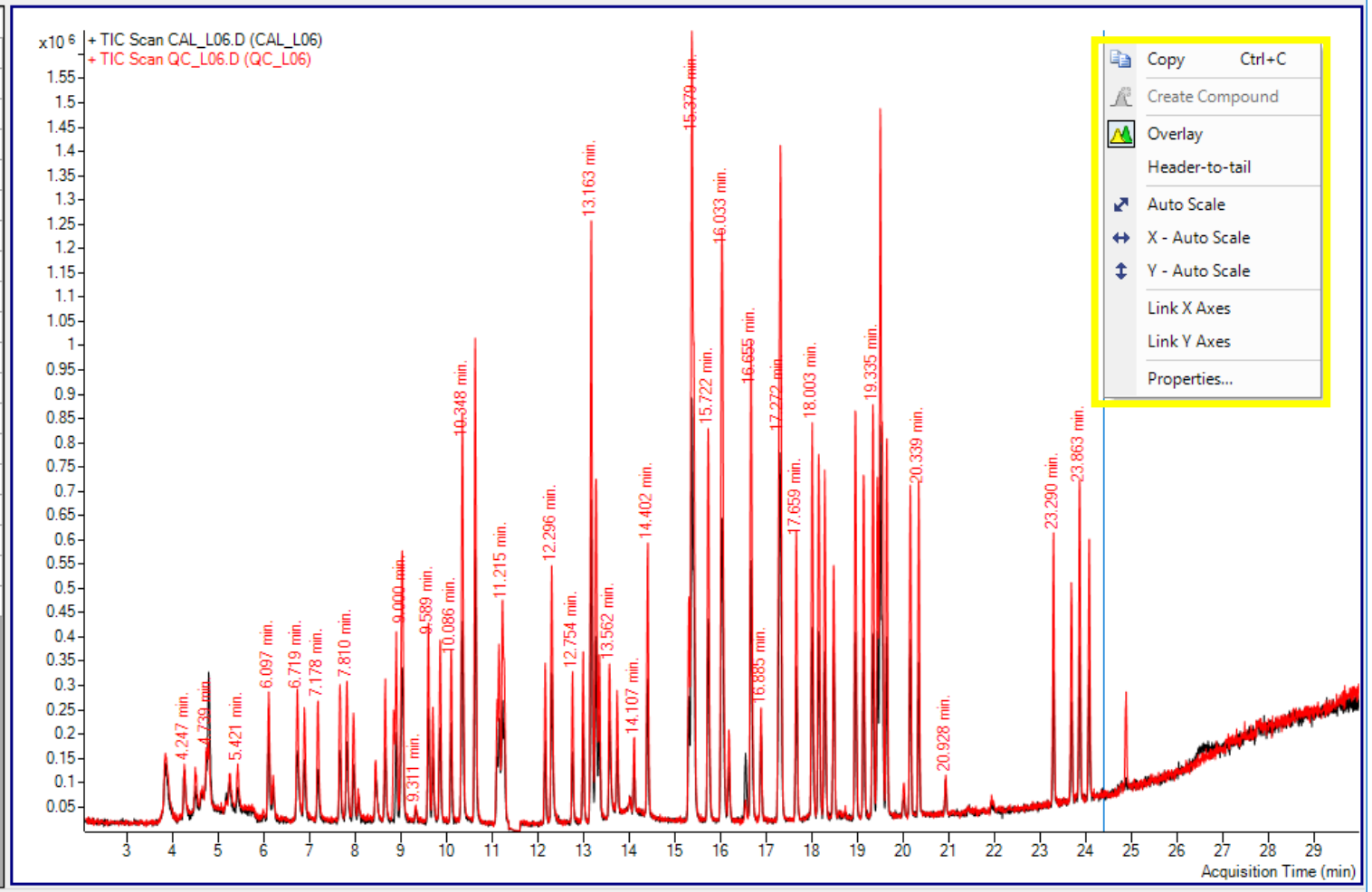
Chromatogram Information

File Edit View

Create Compound | Max # of panes: 2

	Name	Data File	Signal	Color	Anchor
☐	CAL_L03	CAL_L03.D	TIC Scan		☐
☐	CAL_L04	CAL_L04.D	TIC Scan		☐
☐	CAL_L05	CAL_L05.D	TIC Scan		☐
☑	CAL_L06	CAL_L06.D	TIC Scan		☐
☐	CAL_L07	CAL_L07.D	TIC Scan		☐
☐	CAL_L08	CAL_L08.D	TIC Scan		☐
☐	CAL_L09	CAL_L09.D	TIC Scan		☐
☐	CAL_L10	CAL_L10.D	TIC Scan		☐
☐	CAL_L11	CAL_L11.D	TIC Scan		☐
☐	CAL_L12	CAL_L12.D	TIC Scan		☐
☐	CC_L07	CC_L07.D	TIC Scan		☐
☑	QC_L06	QC_L06.D	TIC Scan		☐
☐	Blank01	BLANK01.D	TIC Scan		☐
☐	Blank02	BLANK02.D	TIC Scan		☐
☐	SAMPLE01	SAMPLE01.D	TIC Scan		☐
☐	SAMPLE02	SAMPLE02.D	TIC Scan		☐
☐	SAMPLE03	SAMPLE03.D	TIC Scan		☐
☐	SAMPLE04	SAMPLE04.D	TIC Scan		☐
☐	SAMPLE05	SAMPLE05.D	TIC Scan		☐

Overlay view.
Many other options are available.



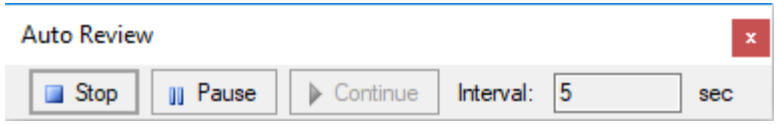
Auto Review

Auto Review Samples

- Displays sample by sample.

Auto Review Compounds

- Displays compound by compound.



- Available in Flat Table or Compound Table.
- Stop, pause, continue and variable intervals.

Demo time

Outlier Check List

Agilent CrossLab
From Insight to Outcome

An **outlier** is a result that is outside the range of acceptable values for a given parameter.

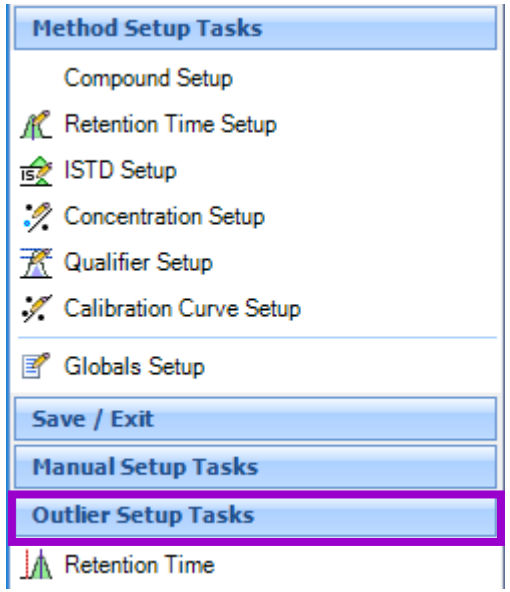
What outliers are important in the workflow?

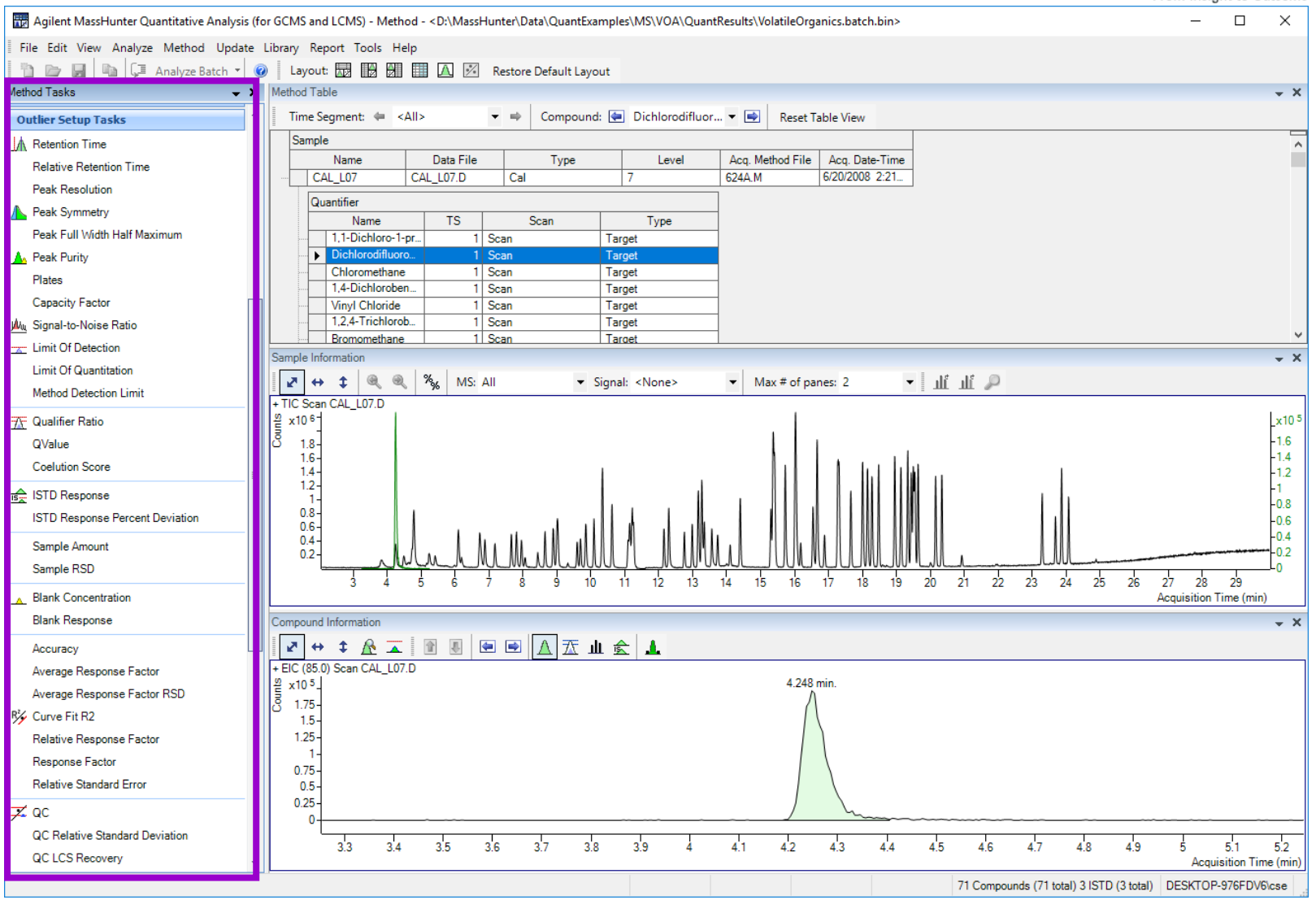
- Retention Time…
- Limit of Detection, Quantitation, Method Detection Limit.
- Qualifier Ratio.
- ISTD Response or ISTD Response Percent Deviation.
- QC, QC Relative Standard Deviation, QC LCS Recovery…
- CC, CC Average Response Factor, CC ISTD Response Ration…
- Matrix Spike, Matrix Spike Percent Recovery….

Outliers Setup Tasks

Agilent CrossLab
From Insight to Outcome

Method > Edit >Outlier Setup Tasks





Outliers are setup in the Method Editor and are part of quantitation method.

Outliers Setup Tasks

Agilent CrossLab
From Insight to Outcome

~ 48 Outliers are available.

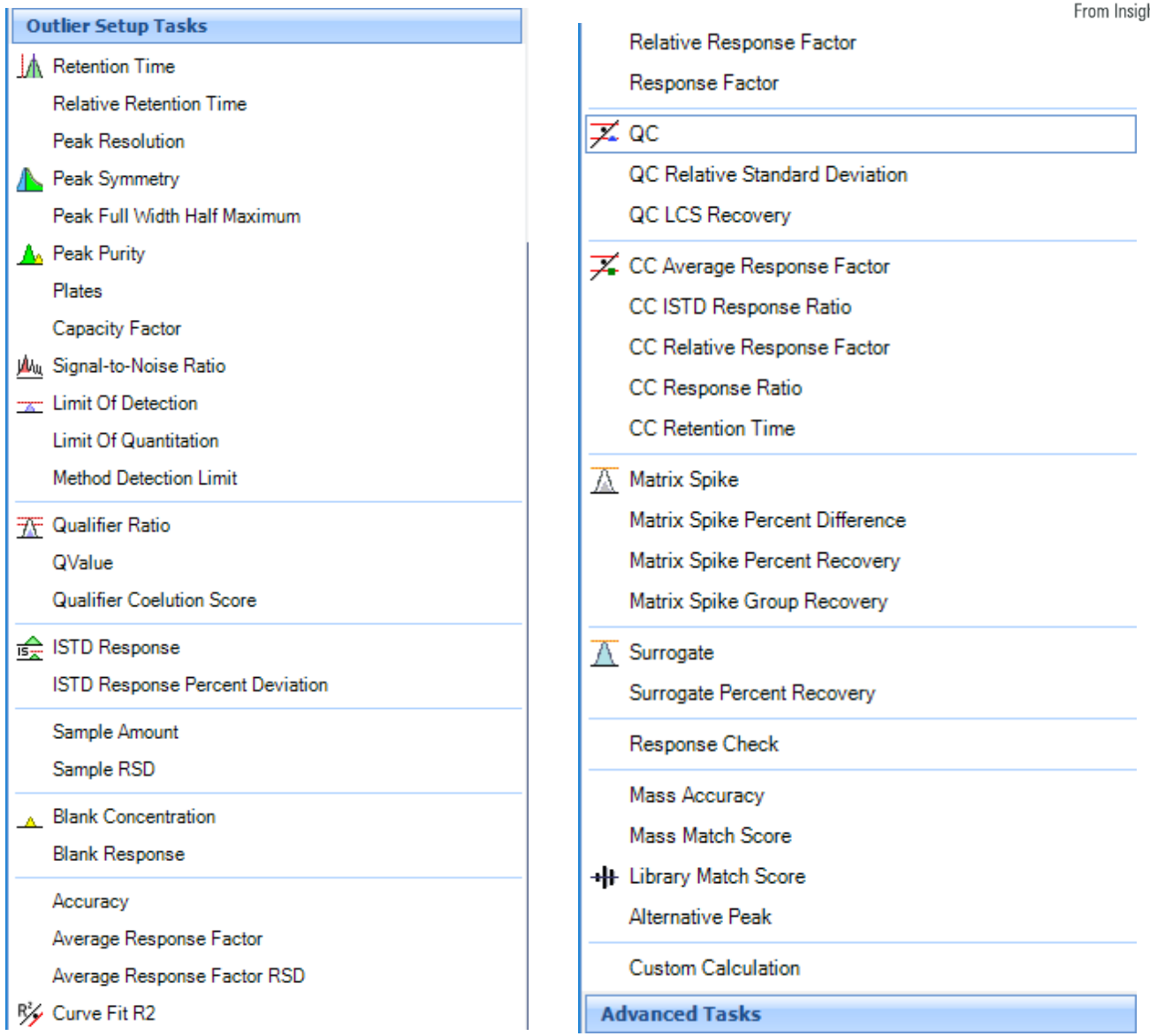
Outliers are not calculated unless values have been set up.

Outliers are used to perform automated quality checks.

Aids in data review by highlighting problem areas.

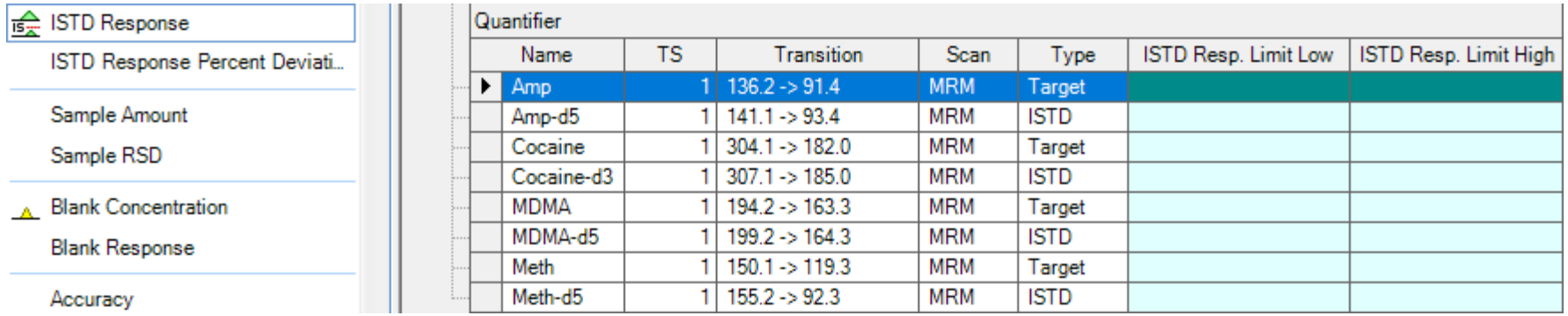
Increases confidence in data integrity by utilizing outliers.

Which outliers are important for my workflow?



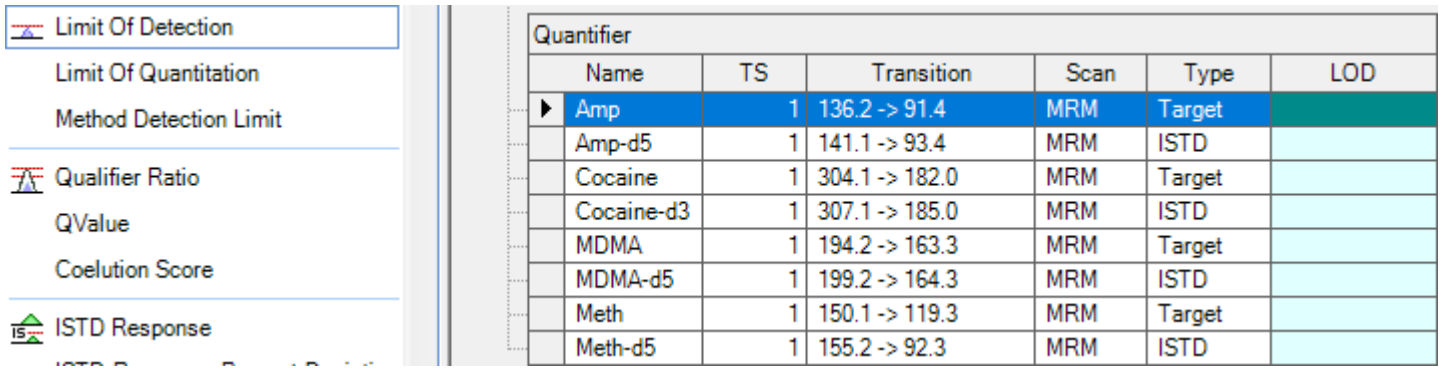
Outliers

- Most Outliers have both a low and high limit.
- Both limits need to be set for the outlier to be calculated.



Quantifier

Name	TS	Transition	Scan	Type	ISTD Resp. Limit Low	ISTD Resp. Limit High
Amp	1	136.2 -> 91.4	MRM	Target		
Amp-d5	1	141.1 -> 93.4	MRM	ISTD		
Cocaine	1	304.1 -> 182.0	MRM	Target		
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD		
MDMA	1	194.2 -> 163.3	MRM	Target		
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD		
Meth	1	150.1 -> 119.3	MRM	Target		
Meth-d5	1	155.2 -> 92.3	MRM	ISTD		



Quantifier

Name	TS	Transition	Scan	Type	LOD
Amp	1	136.2 -> 91.4	MRM	Target	
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	
Cocaine	1	304.1 -> 182.0	MRM	Target	
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD	
MDMA	1	194.2 -> 163.3	MRM	Target	
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	
Meth	1	150.1 -> 119.3	MRM	Target	
Meth-d5	1	155.2 -> 92.3	MRM	ISTD	

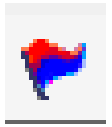
- Some outliers are one dimensional.
- A few only have a single limit.

Outliers
Batch Table

Icons on the toolbar.

Select Outliers

Turn off outlier filter

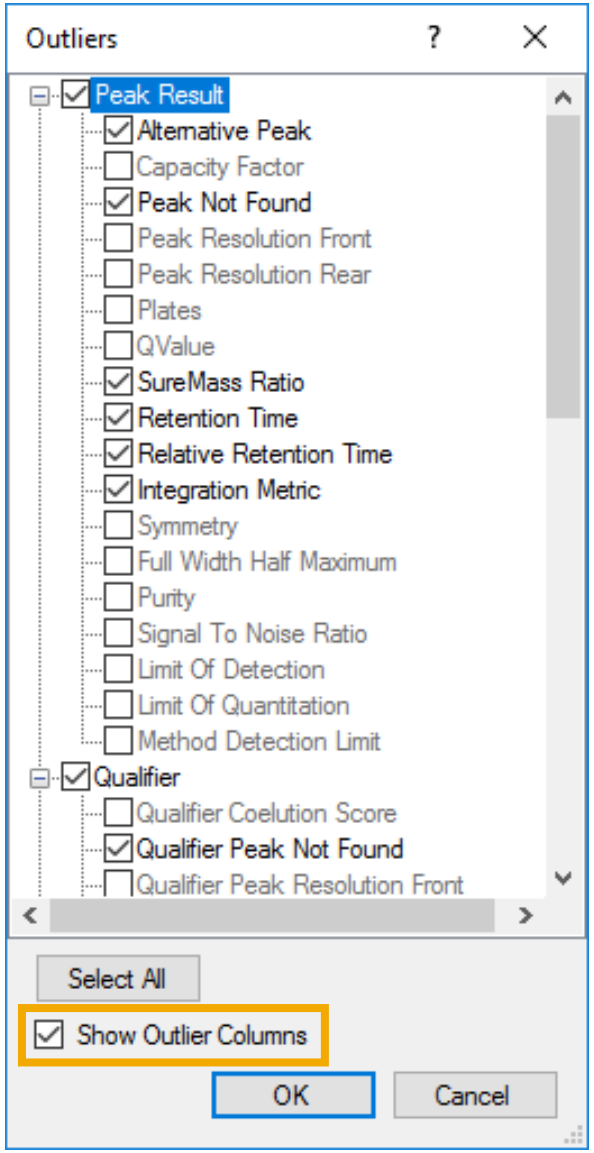


Display rows that have High/Low outliers

Display rows that have High outliers

Display rows that have Low outliers

Display rows that have no outliers



Outliers

Messages and Outliers

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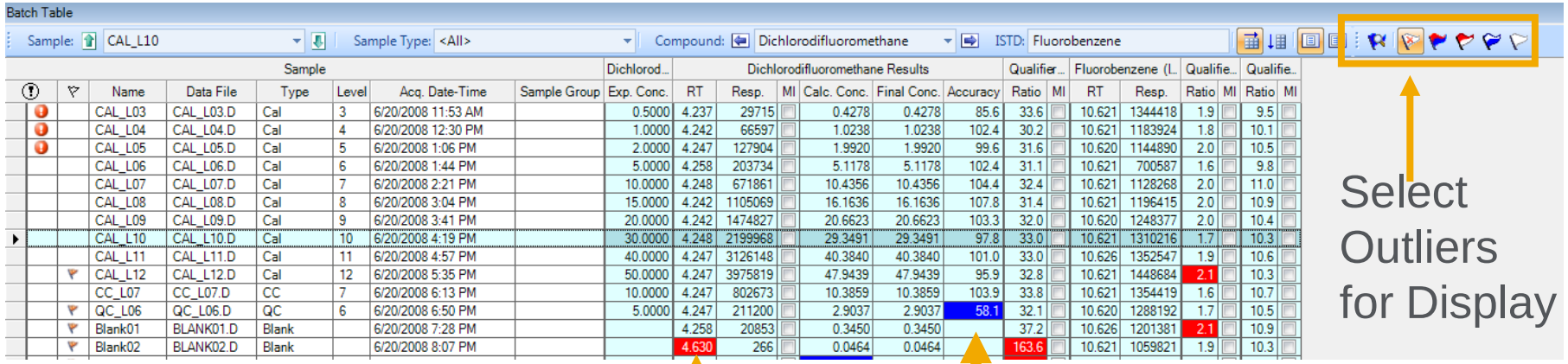
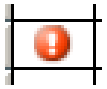
From Insight to Outcome

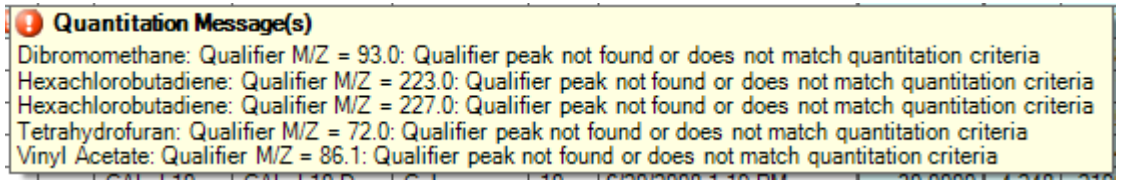


Red Outlier – High (above upper limit)

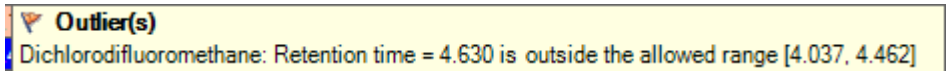
Blue Outlier – Low (below lower limit)



Messages



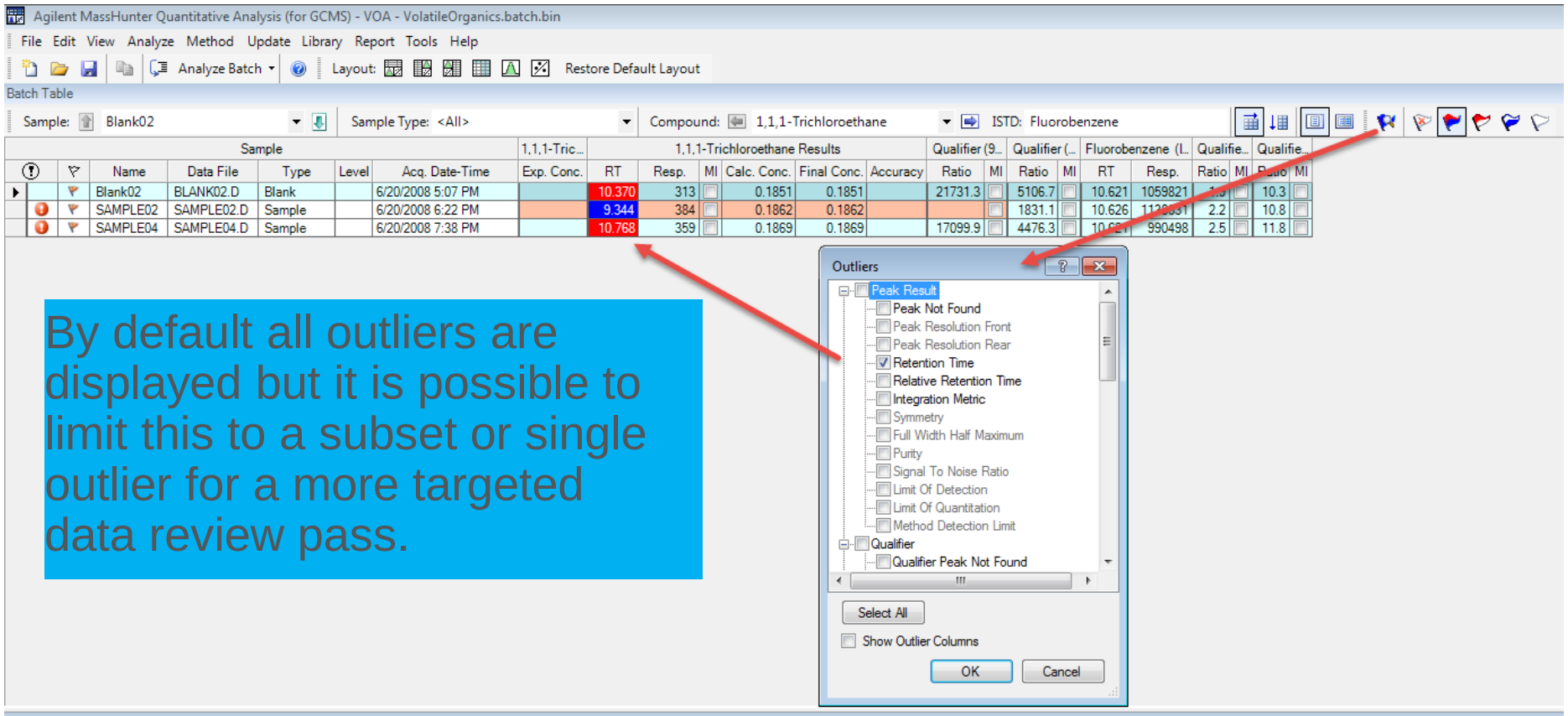
Outliers



Hover cursor over the outlier or message to display details

Outliers

Filter on Outliers in Batch Table



Remember, an outlier is not active unless a limit has been specified for it in the Method Editor.

Outliers

Default outliers

Outlier	Associated Column/Table	Comment
Integration Quality Metric	IntegrationMetricQualityFlags (Peak table)	Defaulted: Using Agile2 Integrator
Qualifier Integration Quality Metric	IntegrationMetricQualityFlags (PeakQualifier table)	Defaulted: Using Agile2 Integrator
Accuracy	Accuracy	Defaulted: +/-20%
Qualifier Ratio	QualifierResponseRatio (PeakQualifier)	Defaulted: +/- 20%
RetentionTime	RetentionTime	Defaulted: +/- 5% relative
Relative Retention Time	RetentionTime	Defaulted: +/- 10% relative

By default these outliers are enabled.

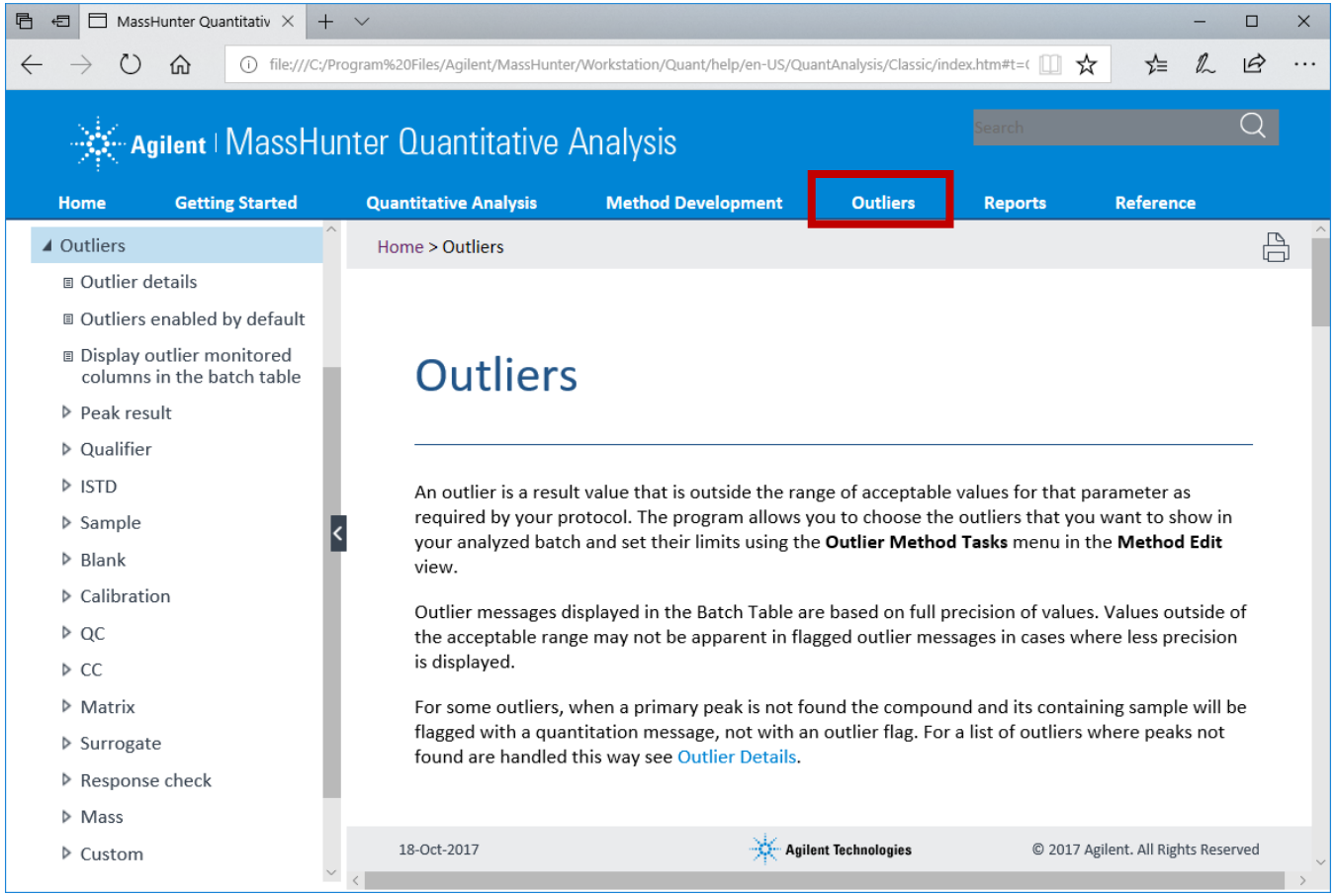
If an outlier is not enabled, it is not calculated.

Accuracy = Calculated/Expected x 100

Outlier
Help

Agilent CrossLab
From Insight to Outcome

New Feature - Help in HTML Format.



Many Outliers also have Quant videos.

Name

- Outlier Criteria Intersection.mp4
- Outliers - Continuing Calibration - CC - Advanced.mp4
- Outliers - FWHM Chromatography - Advanced.mp4
- Outliers - Laboratory Control Spike - Advanced.wmv
- Outliers - Library Match Score - Advanced.wmv
- Outliers - Mass Accuracy - Advanced.wmv
- Outliers - Matrix Spike - Advanced.mp4
- Outliers - Matrix Spike - Introduction - Advanced.wmv
- Outliers - Matrix Spike Demo - Advanced.wmv
- Outliers - Matrix Spike Group Recovery Demo - Advanced.wmv
- Outliers - Matrix Spike Percent Difference Demo - Advanced.wmv
- Outliers - Matrix Spike Percent Recovery Demo - Advanced.wmv
- Outliers - Peak Not Found - Advanced.wmv
- Outliers - Peak Purity - Advanced.wmv
- Outliers - Pharma suitability - Advanced.mp4
- Outliers -Symmetry - Starter.mp4
- Outliers -Symmetry Demo - Starter.mp4

Metric Plot

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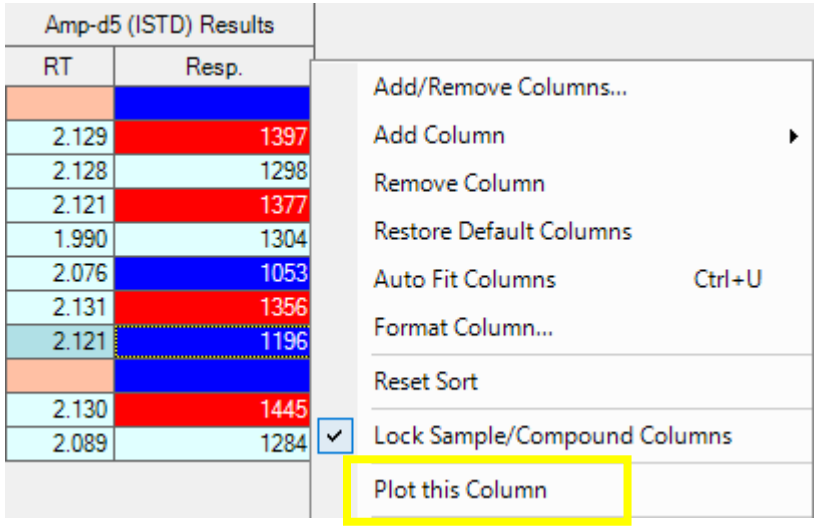
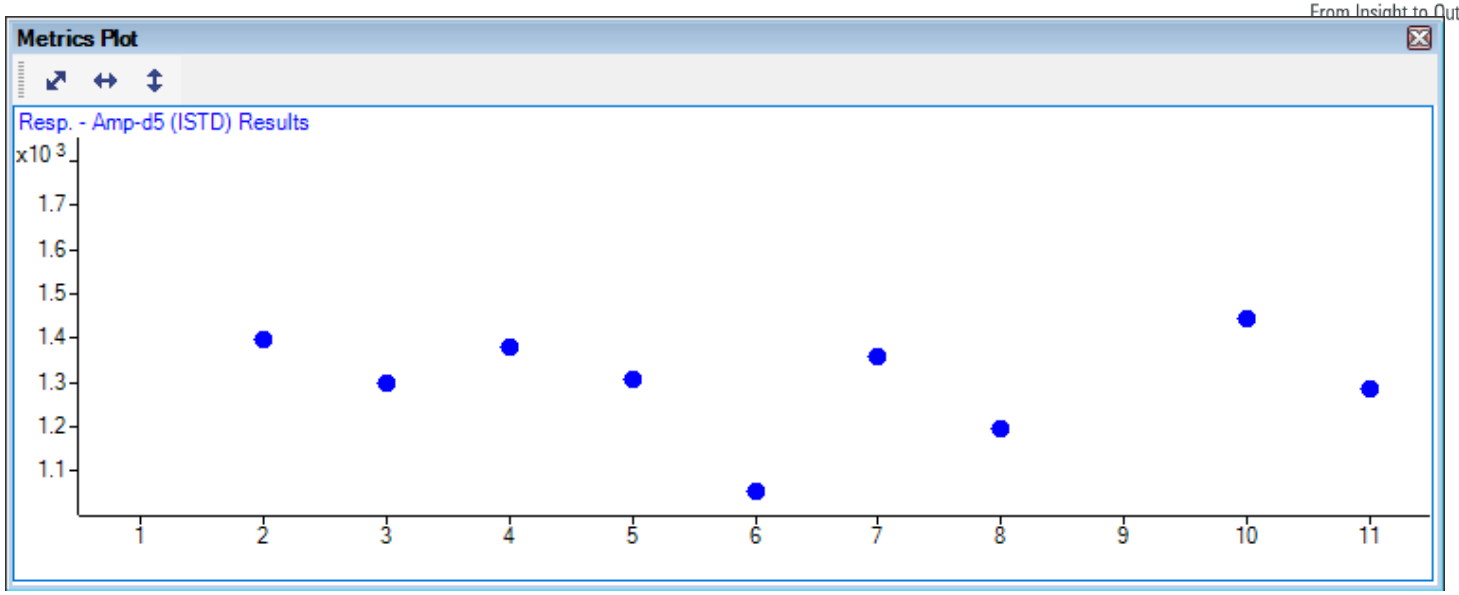
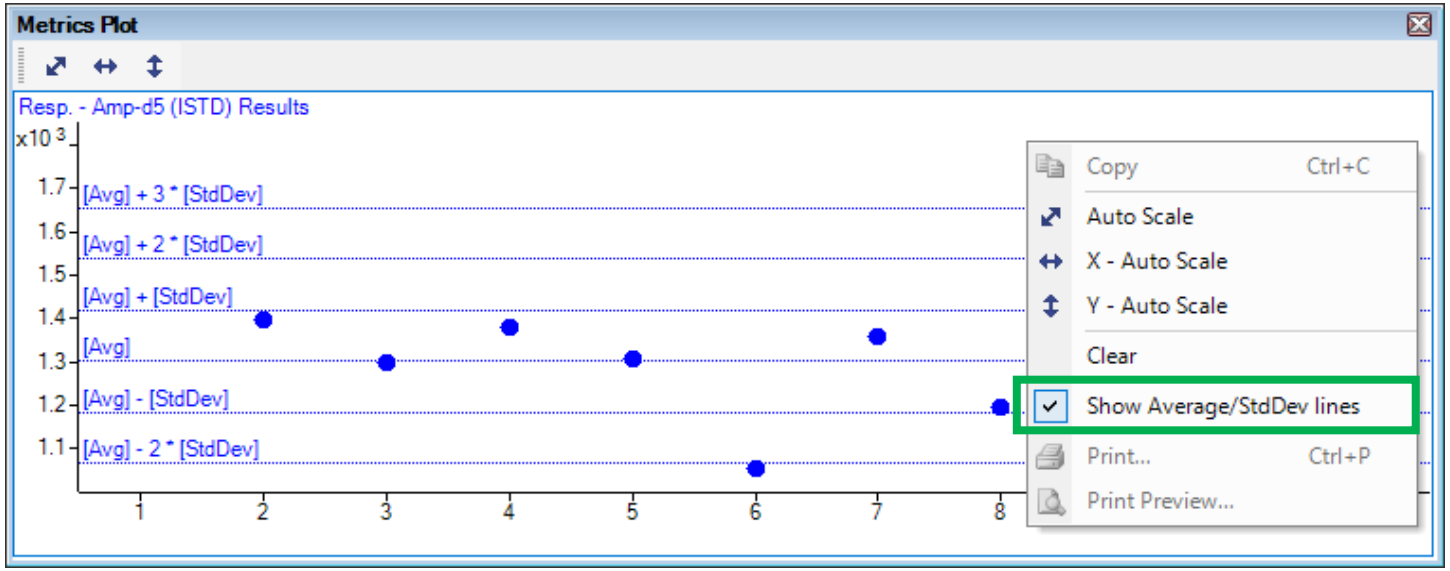
Right click (on header) > Plot this Column.





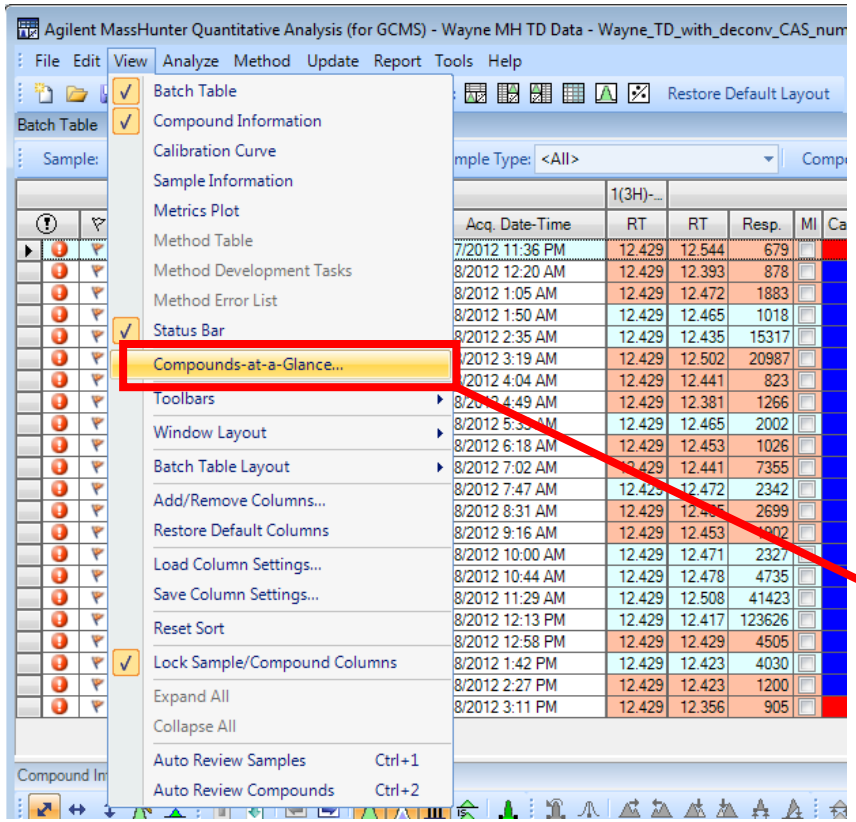
Right click (in the plot window) > Show Average/Std Dev lines.



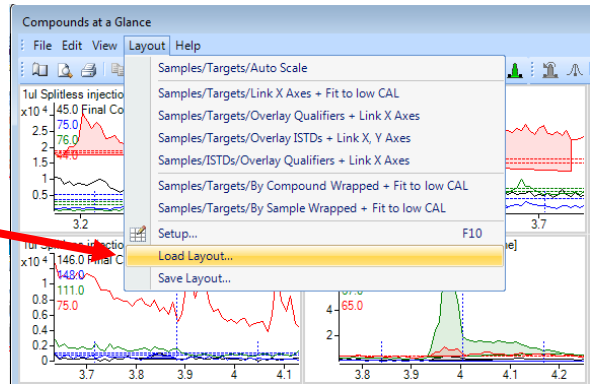
Tip: Use Metric Plot for determining potential problems.

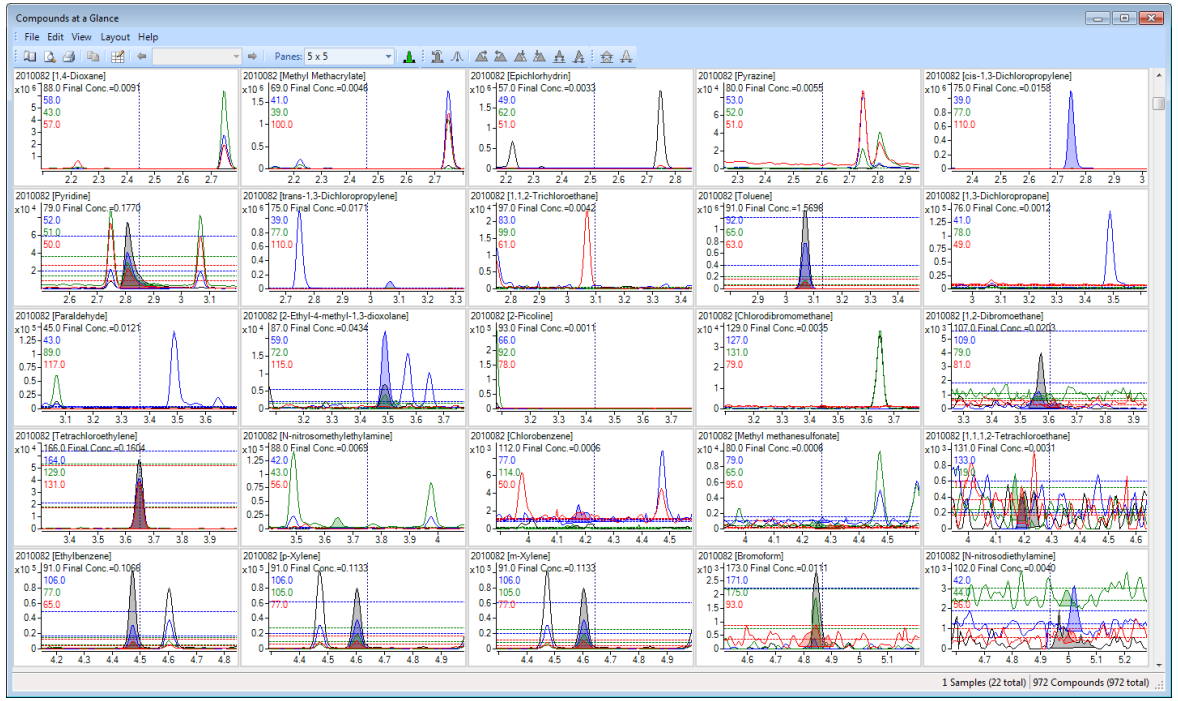
Compounds at a Glance

View > Compounds-at-a-Glance

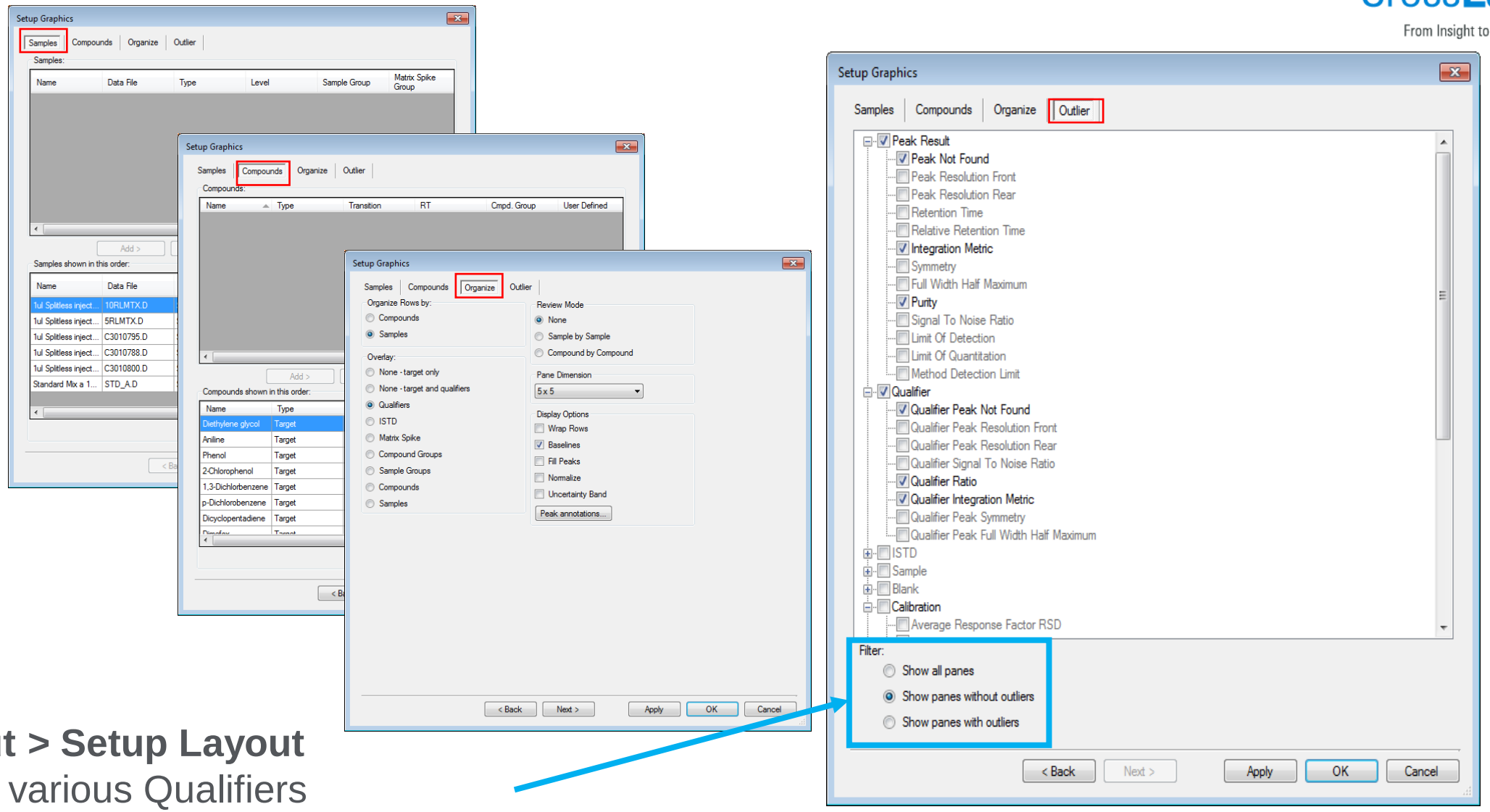


Custom layouts can be loaded and saved.





Compounds at a Glance



Layout > Setup Layout

Select various Qualifiers

Show panes with or without outliers

Compounds at a Glance
Data Review

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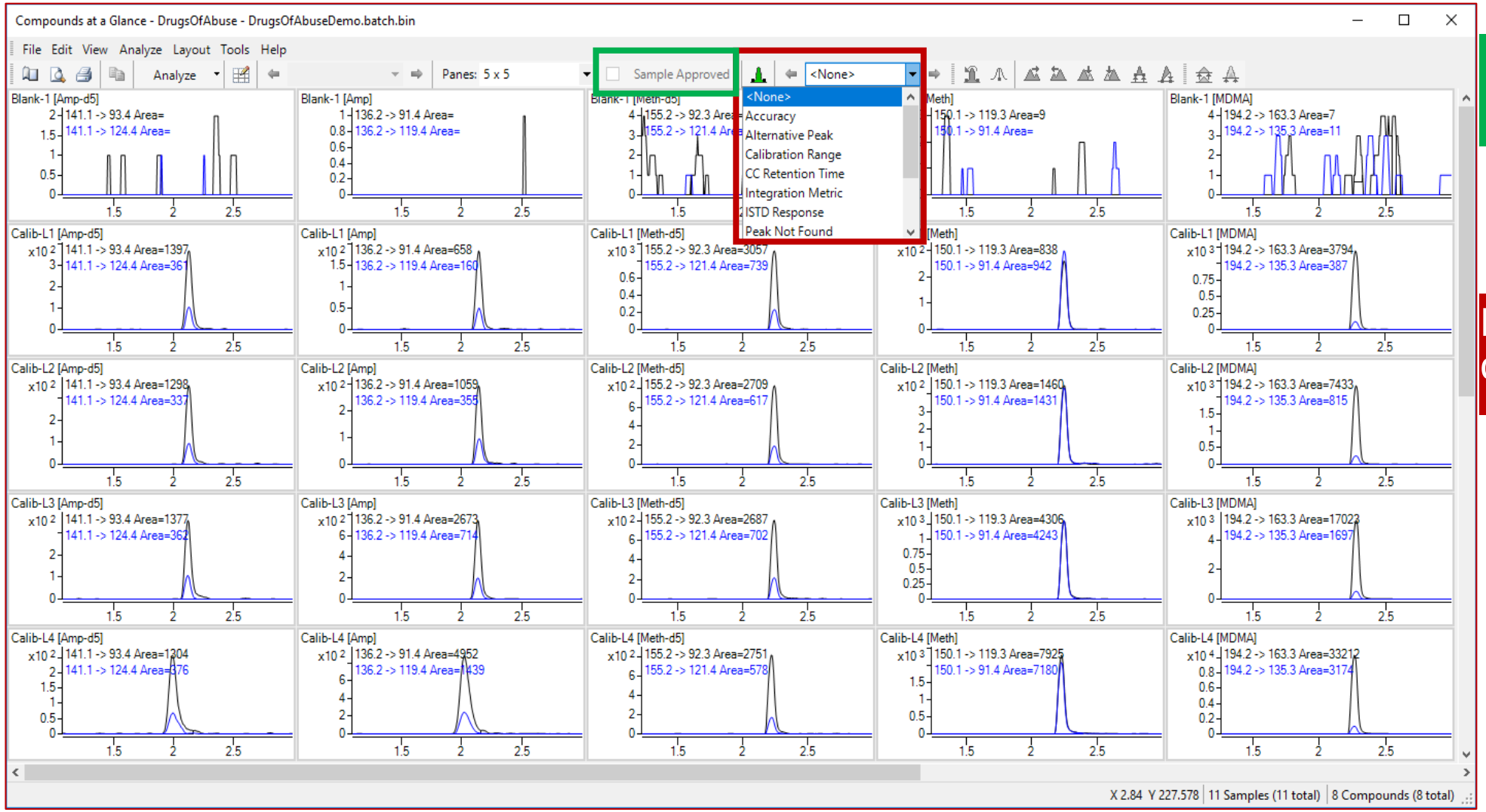

Sample Approved box for QA.

Display outliers by category.

New Feature in Quant B.09.00

Compounds at a Glance
Review Sample by Sample

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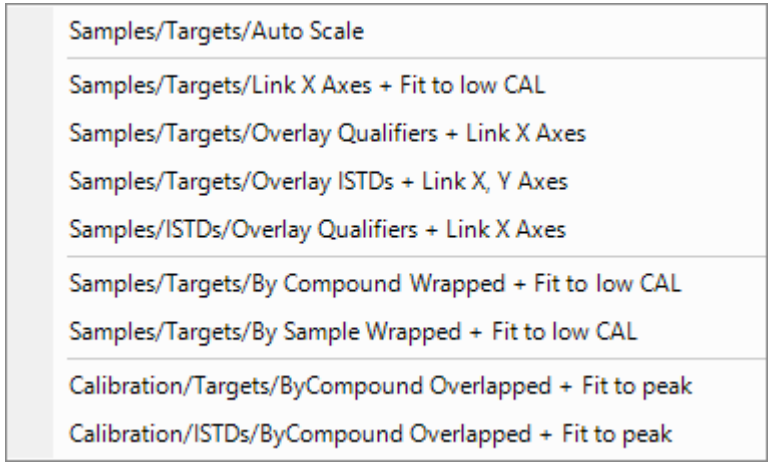
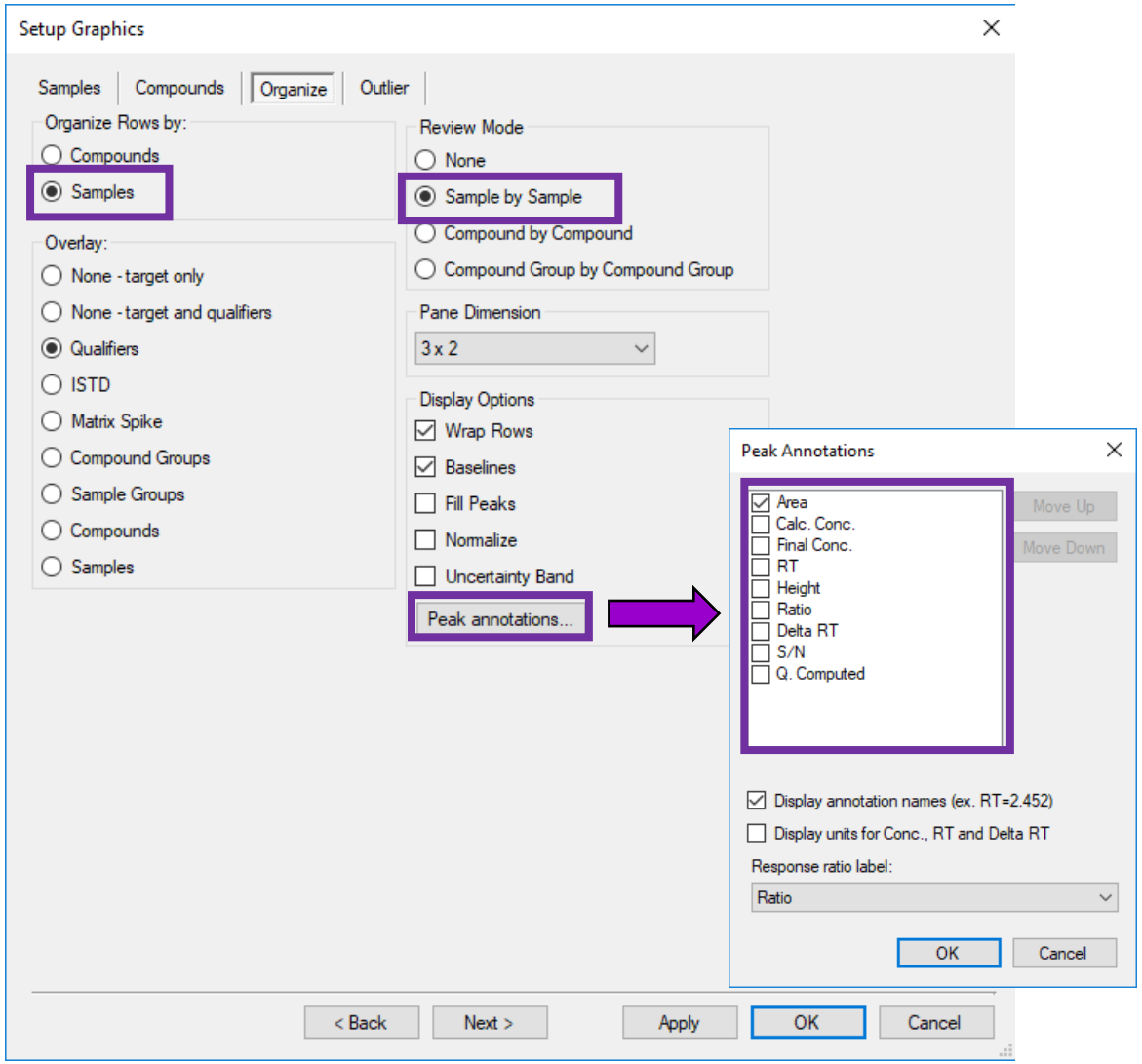
From Insight to Outcome

- Can be reviewed Sample by Sample.
- Optional Pane Dimensions
- Can scroll through the samples.
- Once the layout is saved it can be loaded time after time.
- Numerous Predefined Layouts

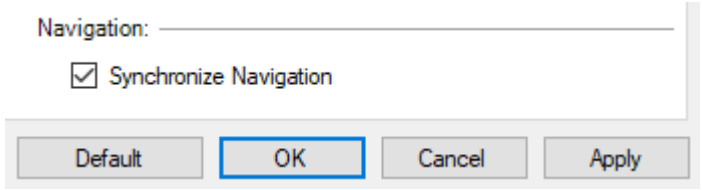




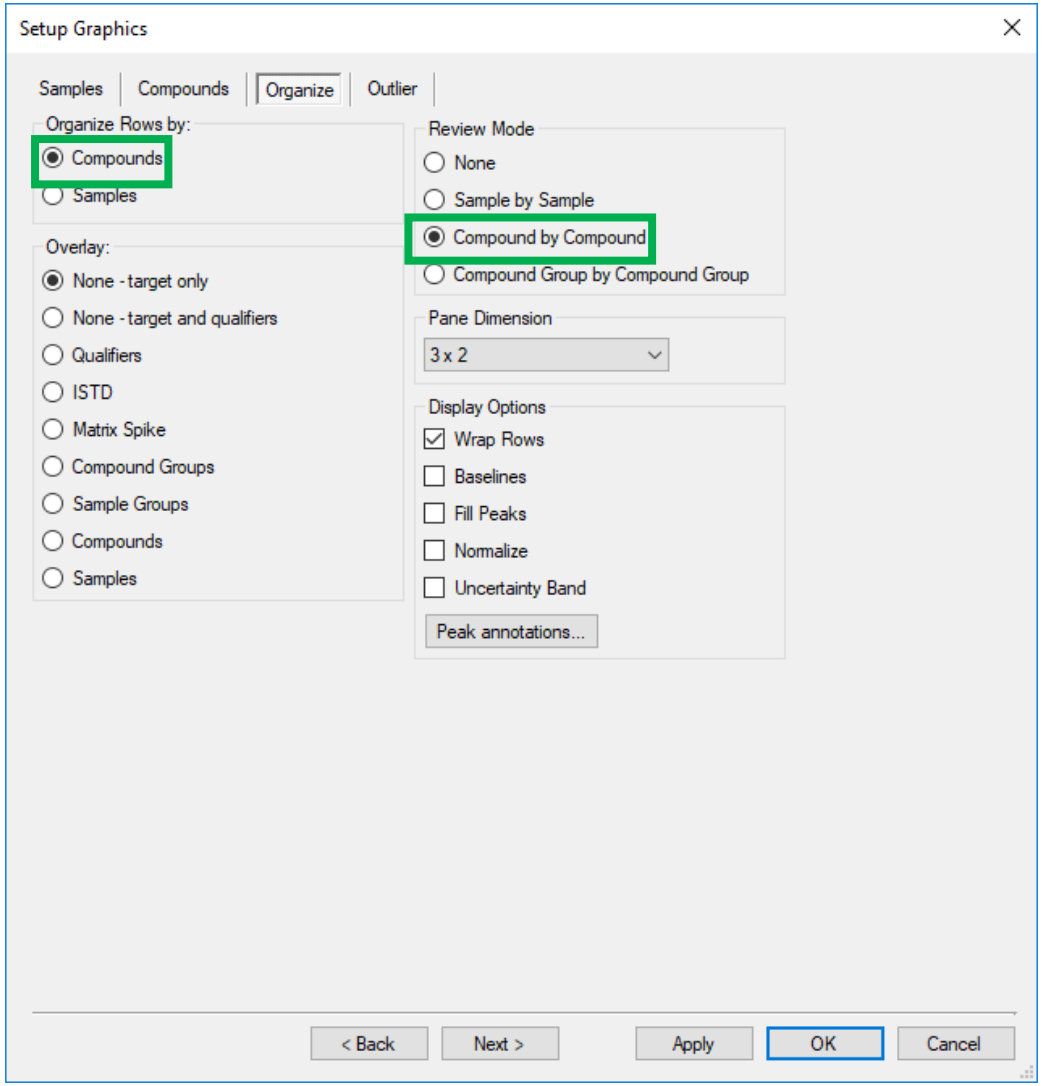
Compounds at a Glance
Review Compound by Compound

Agilent CrossLab
From Insight to Outcome

- Layout > Setup Layout or from 
- Layouts can be customized.
- Can be reviewed Compound by Compound.
- Various Overlay modes.
- Various Display Options.
- Can synchronize Compounds at a Glance with Quantitative Analysis.



- Right click **Properties > Synchronize Navigation** (global parameter).



Demo time

Summary

- Target Compound Analysis
- Quant Method Checklist
  - RT setup and RT Criteria
  - Reference and Non-Reference windows
- Integrators
  - Peak Filters
  - Adjusting Peak Filter Area Thresholds Zero Peak Below LOD
  - Correlation window
- Data Review
  - Filtering on Sample Type, Sample Group and Auto Review
- Outliers
  - ~ 48 outliers are available
  - Which outliers are important to my workflow?
- Compounds at a Glance

Display outliers by category.

Training Resources
Available Training Resources

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From a team of industry experts that deliver a high quality learning experience.

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- Search and find courses that meet your interests and needs in the format they require.

Introduce new eLearning capabilities
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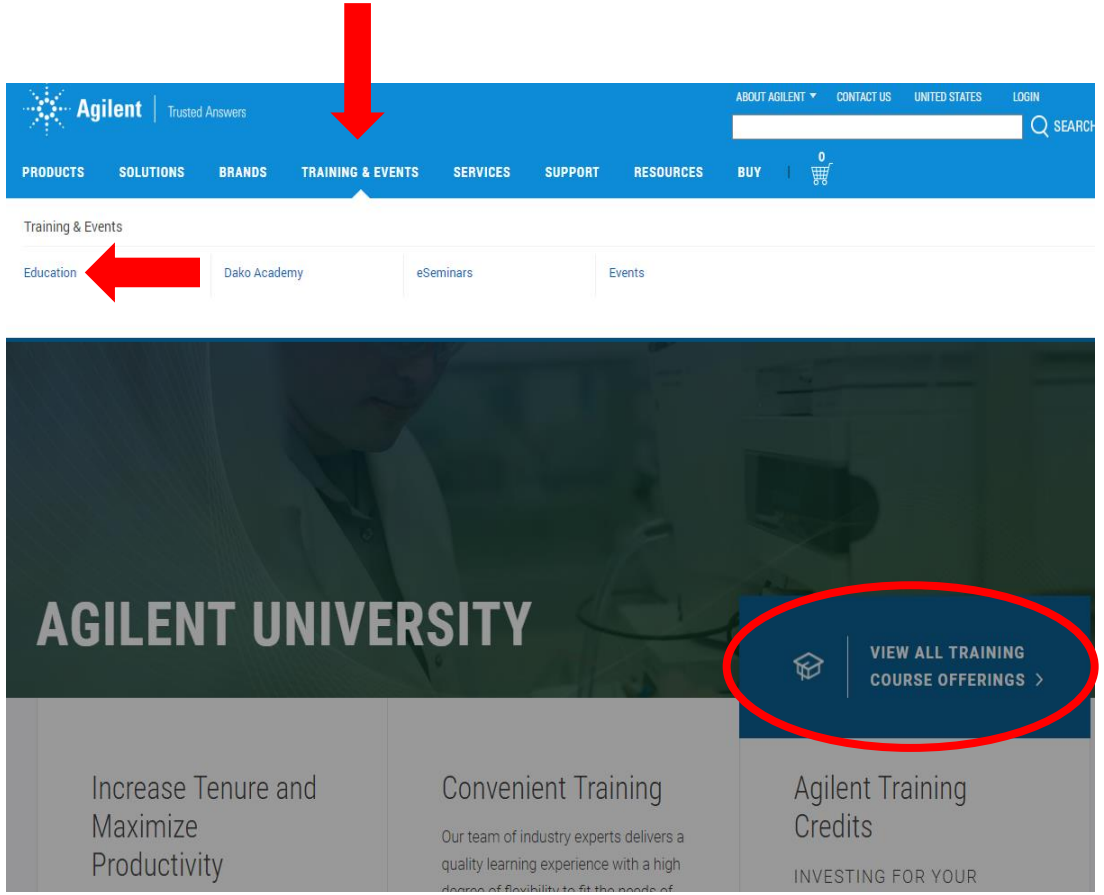
- Foundational subjects
- Intermediate subjects
- Advanced subjects
- Workflow and applications

Helping customers
- Educate your employees on Agilent instruments and software.
- From new hires to the most seasoned scientists.



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